JOURNAL OF DAIRY SCIENCE

Contents

Effect of Forced Exercise on Bull Fertility. J. W. Snyder and N. P. Ralston 125

The Mammary Elimination of Radioiodine. W. E. Wright, J. E. Christian, and F. N. Andrews 131

Milk Lipase. T. W. Albrecht and H. O. Jaynes 137

A Modified Procedure for the Separation of Acidic Amino Compounds Using a Sulfonated Polystyrene Resin. M. K. Hamdy, W. J. Harper, and H. H. Weiser 147

A Comparison of the Fertility of Bull Semen Diluted in Egg Yolk–Citrate and Homogenized Milk. J. R. Perkins, M. C. Carpenter, and D. M. Seath 155

Aluminum in Cow's Milk. J. G. Archibald 159

Proving Dairy Sires and Dams. Jay L. Lush and Lon D. McGilliard 163

Relationship of Size of Growing Cattle to Pasture Intake and Its Use as an Index of Palatability. C. M. Martin, W. F. Brannon, and J. T. Reid 181

Further Studies on the Production of a Hyperkeratosis in Calves with Topically Applied Base-Oils for Use in Livestock Sprays. W. G. Hoekstra, R. J. Dicke, and P. H. Phillips 186

Effect of Antioxidants in the Control of Oxidixed Flavor Development in Stored Frozen Cream. II. Use of Ethyl Hydrocaffeate. A. J. Gelpi, Jr., E. W. Bryant, and L. L. Rusoff 197

The Effect of Semen Dilution with a Yolk-Containing Extender on the Oxygen Uptake and Motility of Bull Spermatozoa. Marcus W. H. Bishop and G. W. Salisbury 202

Studies on Corn Steep Liquor in the Nutrition of Certain Lactic Acid Bacteria. H. E. Kennedy and M. L. Speck 208

The Influence of Some Surfactants upon the Self-Dispersion and Churning of Whole Milk Powder. D. W. Mather and H. A. Hollender 217

Technical Notes 224

People and Events 225

Our Industry Today:

Newer Concepts of Dairy Waste Disposal. R. H. Jackson and G. H. Watrous, Jr. 231

The Value of the Coliform Test Applied to Fruit Flavored Ice Cream. F. W. Barber 233

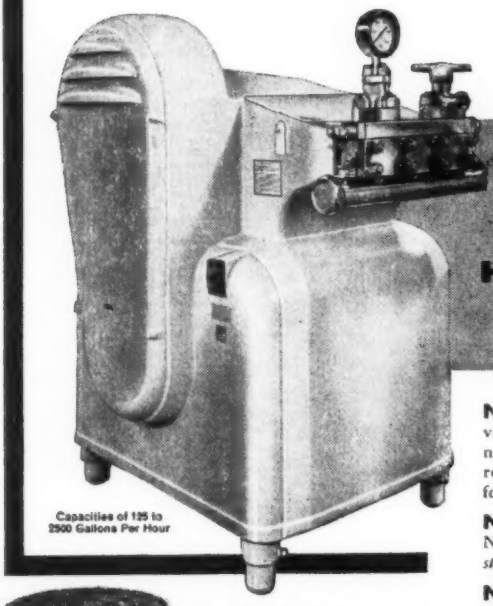
Abstracts of Literature A19

ANNUAL MEETING
JUNE 20-23, '55
EAST LANSING, MICH.

Vol. XXXVIII, No. 2, February, 1955

Published by the

AMERICAN DAIRY SCIENCE ASSOCIATION



Capacities of 125 to 2500 Gallons Per Hour

NEW

MULTI-FLO HOMOGENIZER

FOR MILK OR MIX

New CP Bell-Flow Single Service Valve provides effective break-up and dispersion. Insures new homogenizer performance and uniform results with every run. Replaceable in minutes for pennies. *Exclusive with CP.*

New STAINLESS CONSTRUCTION—Now—all metal in contact with product is *stainless steel.*

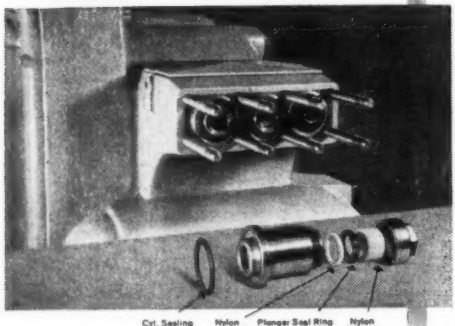
New CP head design employs only *one plunger packing ring* per cylinder. New nylon seal seat and follower reduce maintenance—give longer life at less cost.

New CP clamp-on suction type manifold with plastic seal rings assures positive sealing—saves time.

You get these new features PLUS these time-tested CP Multi-Flo benefits: . . . uniformly fine results on true low pressures with less power, less strain... Flexibility in design—that lets you increase capacity of most models very economically by adding larger components on original frames.

Enjoy the performance and economy features of the new CP Multi-Flo Homogenizer. Write for more complete details, or ask your CP representative.

NEW CP BELL-FLOW SINGLE SERVICE VALVE is shaped and designed to give effective break-up and dispersion.



New CP head design employs just one plunger seal ring per cylinder, with nylon seal seat and follower. Less maintenance, better performance.

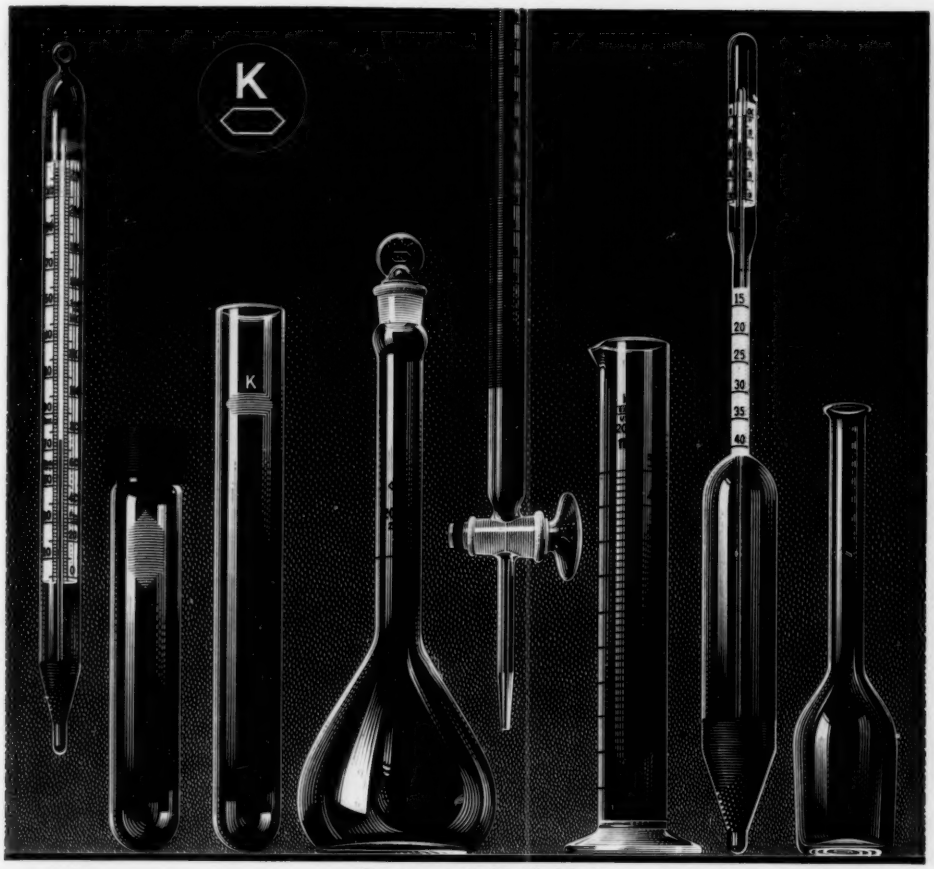
THE *Creamery Package* MFG. COMPANY

General and Export Offices:
1243 W. Washington Blvd., Chicago 7, Illinois

Branches: Atlanta • Boston • Buffalo • Chicago • Dallas • Denver • Houston • Kansas City, Mo. • Los Angeles • Memphis • Minneapolis • Nashville • New York • Omaha • Philadelphia • Portland, Ore. • St. Louis • Salt Lake City • San Francisco • Seattle • Toledo • Waterloo, Iowa

CREAMERY PACKAGE MFG. CO. of CANADA, LTD.
267 King St., West, Toronto 2B, Ontario

THE CREAMERY PACKAGE MFG. COMPANY, LTD.
Mill Green Road, Mitcham Junction, Surrey, Eng.



Precision-made Kimble Glassware—*standard in an exacting industry.*

ACCURATE!

KIMBLE DAIRY TEST WARE

Made to make the most rigid tests of the industry

Outstanding quality and assured accuracy have established Kimble Dairy Glassware as the recognized standard of the dairy industry—for test, analysis and control.

Kimble Dairy Glassware meets all federal and state requirements . . . passes the most rigid tests for accuracy and serviceability. It is made by skilled craftsmen who have devoted a lifetime to the creation of precision scientific instruments.

Uniform machine-made tubing, careful fabrication, correct retempering, durable fused-in calibrations and individual retesting assure long life and accuracy of Kimble ware. To be ACCURATE . . . specify KIMBLE.

Kimble Dairy Glassware is distributed by leading dairy supply houses throughout the United States and Canada.

Kimble Dairy Test Ware is manufactured by Kimble Glass Company, subsidiary of Owens-Illinois

KIMBLE DAIRY TEST WARE
AN (O) PRODUCT

OWENS-ILLINOIS
GENERAL OFFICES • TOLEDO 1, OHIO

Your advertisement is being read in every State and in 45 Foreign Countries

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION

Published at
119-123 WEST PARK AVENUE, CHAMPAIGN, ILLINOIS

P. H. TRACY, *Editor*
Urbana, Illinois

Editorial Board

J. O. ALMQUIST State College, Pa.	ROBERT JENNESS St. Paul, Minn.	E. E. ORMISTON Urbana, Ill.	M. L. SPECK Raleigh, N. C.
F. J. DOAN State College, Pa.	J. J. JEZESKI St. Paul, Minn.	R. L. PERRY Berkeley, Calif.	R. W. TOUCHBERRY Urbana, Ill.
R. E. ELY Washington, D. C.	J. P. MIXNER Sussex, N. J.	J. T. REID Ithaca, N. Y.	EVERT WALLENFELDT Madison, Wis.

Committee on Journal Management
J. H. ERB, *Chairman*
G. W. TRIMBERGER — P. H. TRACY (ex officio)
W. M. ROBERTS — T. D. HARMAN (ex officio)

Manuscripts for publication in the Journal should be submitted to the editor, P. H. Tracy, Department of Food Technology, University of Illinois, Urbana. See Vol. 37, January issue, page 103 for information concerning the preparation of manuscripts.

Subscriptions. Price, $15.00 per volume in North and South America; $15.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to T. D. Harman, The Ohio State University, Columbus 10, Ohio.

Subscriptions for the British Isles and British Empire, except for Canada and Australia, should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert St., St. Leonards, N. S. W., Australia.

Membership dues in the Association are $10.00 per year.

Advertising copy should be mailed to T. D. Harman, The Ohio State University, Columbus 10, Ohio. Advertising plates or cuts should be mailed direct to The Garrard Press, 119-123 West Park Ave., Champaign, Illinois.

Post office notices of undeliverable copies and changes of address should be sent to T. D. Harman at the address above stated.

OFFICERS OF THE ASSOCIATION

L. A. MOORE, *President*, Beltsville, Md. — T. D. HARMAN, *Sec.-Treas.*, Columbus, Ohio
I. A. GOULD, *Vice-President*, Columbus, Ohio — P. H. TRACY, *Journal Editor*, Urbana, Illinois

DIRECTORS

N. N. ALLEN Madison, Wis.	R. E. HODGSON Beltsville, Md.	GEORGE HYATT, JR. Raleigh, N. C.	D. V. JOSEPHSON State College, Pa.
W. V. PRICE Madison, Wis.	L. H. RICH Logan, Utah	G. W. SALISBURY Urbana, Ill.	

Entered as second-class matter at the post office at Champaign, Illinois, under the act of March 3, 1879.

Your advertisement is being read in every State and in 45 Foreign Countries

Pipelines stay UP... cleaning costs go WAY DOWN with CHERRY-BURRELL C-I-P Fittings

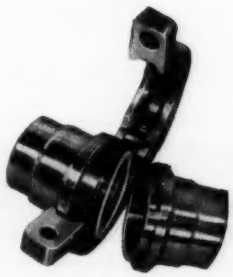
You Can Cut Clean-up Time Up to 70% and save on both installation and maintenance with new Cherry-Burrell stainless steel Cleaned-In-Place (C-I-P) Pipelines and Fittings.

Cleaner Lines in Less Time: No time lost taking down lines. They're cleaned "in place" with high-velocity, uniform hydraulic scrubbing. No recontamination from airborne bacteria or from handling.

Save Installation Time and Cost: Fewer parts to handle. 21-foot pipe sections eliminate need for many unions. Vise-like strength of Cherry-Burrell union does away with pipe hangers and supports.

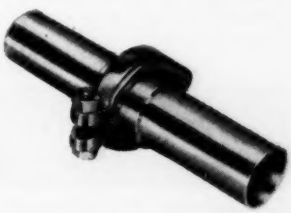
Less Maintenance: Lines not frequently dismantled are less likely to be dropped, dented, scored.

CHERRY-BURRELL C-I-P CLAMP FORMS VISE-LIKE UNION

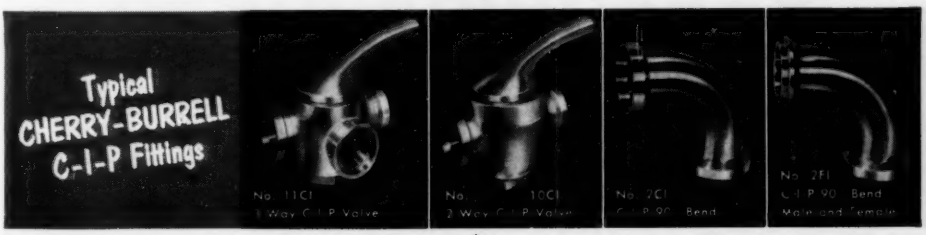


1 Self-centering "Teflon" gasket placed in female ferrule. "Teflon" gasket is non-toxic, non-absorbent, impervious to fats, acids and alkali.

2 Male and female ferrules assembled with gasket in place. Matching 37° clamping angle on ferrules and clamps. Ferrule seats machined to close tolerance. Gasket cannot get out of alignment, swell or creep.



3 Ferrules with metal-to-metal stop form union with predetermined squeeze point on gasket. No cracks, crevices or extrusions to harbor bacteria. Union will contain pressures up to 250 p.s.i.

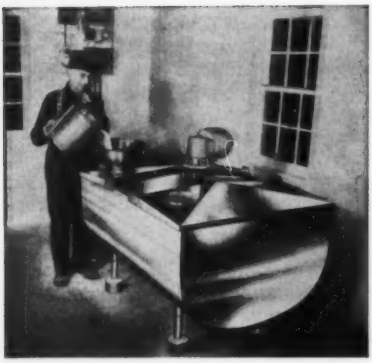


Available in 1½", 2", 2½" and 3" sizes.

Your Cherry-Burrell Representative can show you how C-I-P Lines and Fittings can save money in any plant. Write for Bulletin G-470.

CHERRY-BURRELL CORPORATION
427 W. Randolph Street, Chicago 6, Ill.
Equipment and Supplies for Industrial and Food Processing
FACTORIES, WAREHOUSES, BRANCHES, OFFICES OR DISTRIBUTORS AT YOUR SERVICE IN 56 CITIES

Your advertisement is being read in every State and in 45 Foreign Countries



SAVE EFFORT

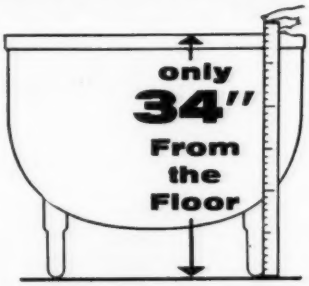
with the

Mojonnier

LOW DESIGN

BULK COOLER

- Mojonnier Low Design Bulk Coolers save labor and effort in the milk house. The actual floor-to-tank top dimension of the 300 gallon unit (illustrated above) is only **34 inches**, reducing pouring effort. On the 100, 150 and 200 gallon Bulk Coolers, this height is only **32¼ inches** from the floor.
- Mojonnier Bulk Coolers are of rounded bottom construction for easy cleaning, inside and out, and the all stainless steel construction helps too.
- Milk is quickly cooled to 34 - 36°F, at lowest possible power cost.
- Decide to enjoy the advantages of the convenient, efficient Mojonnier Low Bulk Cooler. Bulletin 290 gladly sent upon request.



MOJONNIER BROS. CO. 4601 W. OHIO ST., CHICAGO 44, ILLINOIS

Mojonnier BULK MILK SYSTEM

Now Available . . .

a limited supply of the new

DECENNIAL INDEX

of the JOURNAL OF DAIRY SCIENCE

Covering Volumes XXI-XXX. All Copies Clothbound.

COST: Members, $6.50 Non-Members, $7.50

Send check or money order to the

AMERICAN DAIRY SCIENCE ASSOCIATION

Ohio State University **Columbus 10, Ohio**

Your advertisement is being read in every State and in 45 Foreign Countries

JOURNAL OF DAIRY SCIENCE

VOLUME XXXVIII FEBRUARY, 1955 NUMBER 2

EFFECT OF FORCED EXERCISE ON BULL FERTILITY[1]

J. W. SNYDER[2,3] AND N. P. RALSTON

Dairy Department, Michigan State College, East Lansing

Bulls in artificial breeding association studs that have proved their ability to sire high producing and good type daughters need to be kept in service as long as possible. Whether or not exercise is beneficial to a bull's fertility and longevity has long been a controversial issue. The question arises as to how much and what kind of exercise mature bulls should receive. The method and amount of exercise a bull gets vary widely between individual bulls and between studs.

Bartlett and Perry *(1)* reported that three bulls exercised at the rate of 2½ miles per hour for 1 hour each day for 1 month showed an increase in semen volume of 51% as compared to the previous months. Hamilton and Symington *(3)* observed that daily exercise increased the volume of the ejaculate. These authors observed an increased motility and greater sperm concentration when exercise was provided. Woodward *(8)* reported that exercise at the rate of 2½ miles per hour for 30 minutes daily greatly decreased the interval of time required to obtain a semen sample and increased motility and longevity of the spermatozoa. Weatherby and associates *(7)* ascribed the lower quality of semen obtained during the months of February and March to the lack of exercise. On the contrary, Lepard *et al. (4)* noted no significant differences in the semen of exercised and nonexercised bulls in morphology, concentration, and survival time of spermatozoa at 4.5° C. The volume of semen and conception rates from exercised bulls were somewhat greater, but of insignificant advantage.

Since the above investigations were conducted for relatively short periods and with few animals, and since few data were given on the fertility of the bulls, this study was designed to include several animals and to cover a 6-month period. The objective of this experiment was to observe the effects of forced exercise of dairy bulls upon their semen characteristics (volume, total spermatozoa number, motility, and percentage and kinds of abnormal spermatozoa) and on the percentage of nonreturns of all services on a 60- to 90-day basis.

Received for publication March 10, 1954.

[1] Published with the approval of the director of Michigan Agricultural Experiment Station as Journal Article No. 1616.

[2] This article is part of a dissertation presented to the faculty of the Graduate School of Michigan State College in partial fulfillment of the requirements for the degree of Master of Science.

[3] Present address: Kansas State College, Manhattan.

EXPERIMENTAL PROCEDURE

Bulls used and experimental design. Thirty-two bulls owned by the Michigan Artificial Breeders Cooperative, Inc., East Lansing, were used. The bulls (varying in age from 4 to 15 years) were divided into two groups of 16 bulls each according to breed (Holstein and Guernsey) and the previous year's 60- to 90-day nonreturn information. Each of these groups was divided into high and low fertility subgroups within breeds and assigned at random to treatment groups.

Feeding and management practices. Bulls within treatment groups were randomized and half of the bulls were housed in 9 ft. × 11 ft. box stalls and the other half in 5 ft. × 8 ft. tie stalls. All bulls received a ration consisting of a 15.8% protein grain mix and an average quality alfalfa-brome grass hay. In addition, grass silage was fed for the first 122 days of the experiment, followed by corn silage for the last 51 days. The average daily level of feeding was 7 lb. of grain mixture, 24 lb. of silage, and 30 lb. of hay.

During the first 35 days, half of the bulls (four each of Holstein and Guernsey sires with high fertility and four each of Holstein and Guernsey sires with low fertility) were exercised for 15 minutes in the afternoon, except on Sunday, on a mechanical exerciser with a speed of 1.24 m.p.h. The amount of daily exercise was increased to 23 minutes at the same rate of speed for the following 14 days. For the remaining 119 days of the experiment these bulls were exercised daily, except Sunday, on the mechanical exerciser for 30 minutes at the rate mentioned above. During this same period the corresponding half of the bulls were housed in 9 ft .× 11 ft. box stalls and 5 ft. × 8 ft. tie stalls.

Weights of the individual bulls were taken at 6-week intervals during the experiment. Each bull was weighed in the morning so as to reduce the effect of feed consumption on body weight. During the 6-month experimental period, close observations were made on each bull to detect any noticeable changes in appetite and general activity.

Semen collection and evaluation. The original plan called for weekly semen collections. This schedule could not be followed strictly because of conditions of the bulls, and some bulls would not mount the teaser cow to serve the artificial vagina. At each collection time, most bulls were ejaculated twice, but in no case was a third ejaculate obtained.

After collection, each ejaculate was taken immediately to the laboratory and examined for gross abnormalities, for volume, for initial motility, and for concentration, with the Cenco photoelectric colorimeter.

Smears were made of the fresh semen from each bull to determine the percentage and kinds of abnormal spermatozoa. The following technique was used: 1. A small sterile stirring rod was carefully introduced into the semen vial to transfer a small drop of semen to a 2-ml. capacity test tube containing 0.5 ml. of physiological saline. 2. The contents of the test tube were then poured on the entire surface of a microscopic slide and allowed to dry at laboratory temperature. The Eosin-Hematoxylin staining method of Gradwohl *(2)* was used.

3. Spermatozoa were classified into the following groups suggested by Salisbury and associates *(5)*: *(a)* morphologically normal; *(b)* true abnormals, which included such types as those possessing any abnormality with respect to the shape or size of head, those with enlarged abaxial, beaded or filiform middle pieces, and those with thickened or doubled tails; *(c)* tailless spermatozoa; *(d)* spermatozoa with broken necks; and *(e)* spermatozoa with coiled tails. With a bacteria counter and a standard technique of moving the slide, 250 spermatozoa were counted on each microscopic slide. The number and types of normal and abnormal spermatozoa were recorded and these figures multiplied by four to give the number of abnormal spermatozoa per 1,000.

Semen used and fertility. Ejaculates with a spermatozoa concentration of 800,000 or above per cubic millimeter and with an initial motility of 70% or above were further processed by diluting with yolk–citrate at the ratio of one part of semen to 30 to 40 parts of diluent. Sixty days after the start of the experiment a combination of 1,000 units each of penicillin and streptomycin was added per milliliter of diluent. After dilution, the semen was gradually cooled to 5° C. at the rate of 15° C. per hour. The diluted semen was poured into 8-ml. vials to within ½ in. of the top. The vials were stoppered with paraffin-coated corks. The percentage of nonreturns was calculated from 33,292 services on a 60- to 90-day nonreturn basis for all services.

Libido. The degree of libido was evaluated by noting the length of time in minutes, or a fraction thereof, that elapsed from the time the bull was 2 or 3 feet from the teaser animal until the completion of service into the artificial vagina. In a few instances bulls were not allowed to mount the teaser animal immediately. The time that a bull was restrained by the handler was not recorded.

RESULTS AND DISCUSSION

Semen characteristics. Each of the 853 semen ejaculates, 453 from the exercised bulls and 400 from the nonexercised bulls, was studied for volume, total spermatozoa number, initial motility, and percentage and kinds of abnormal spermatozoa (Table 1).

By means of the missing plot technique, an analysis of variance of the variations in semen characteristics was made, Snedecor *(6)*. No significant differences were found between the exercised and nonexercised bulls, between the high fertility exercised and nonexercised bulls, or between the low fertility exercised and nonexercised bulls. This was also true between the Holstein exercised and nonexercised sires, between the Guernsey exercised and nonexercised sires, and between breeds.

These data confirm the work of Lepard and associates *(4)*. The findings of this study disagree with those of Bartlett and Perry *(1)*, Hamilton and Symington *(3)*, Weatherby and associates *(7)*, and Woodward *(8)*.

Nonreturn percentages. To determine whether exercised bulls had a higher conception rate on a 60- to 90-day nonreturn basis than nonexercised bulls, 33,292 breeding records were summarized from the herds owned by the patrons of the cooperative. Of these 33,292 services, 17,872 were with semen from the

exercised bulls, and 15,420 with semen from the nonexercised bulls. Table 2 shows the average nonreturn rates by months, treatments, fertility levels, and breeds. An analysis of variance made with the missing plot technique was used in determining significance, Snedecor *(6)*.

TABLE 1

The average values obtained during the 6-month investigation of the semen characteristics

	Exercised bulls (16) [a]				Nonexercised bulls (16)			
	High fertility (8)		Low fertility (8)		High fertility (8)		Low fertility (8)	
	Holstein (4)	Guernsey (4)	Holstein (4)	Guernsey (4)	Holstein (4)	Guernsey (4)	Holstein (4)	Guernsey (4)
No. of ejaculates	151	118	89	95	124	95	91	90
Volume/ ejaculate *(ml.)*	5.9	6.2	7.7	6.7	6.0	5.6	8.0	8.1
Total spermatozoa/ ejaculate *(billions)*	7.9	7.7	8.2	6.9	8.2	7.1	8.4	10.1
Initial motility *(%)*	75.5	77.0	64.7	68.8	75.4	73.1	74.3	69.8
Abnormals *(%)*	10.3	11.7	13.2	18.6	14.2	15.6	15.2	15.2

[a] Number of bulls in each group.

TABLE 2

The average weighted nonreturn values by months, treatments, fertility levels, and breeds[a]

	Exercised bulls (16) [b]				Nonexercised bulls (16)			
	High fertility (8)		Low fertility (8)		High fertility (8)		Low fertility (8)	
	Holstein (4)	Guernsey (4)	Holstein (4)	Guernsey (4)	Holstein (4)	Guernsey (4)	Holstein (4)	Guernsey (4)
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
October	73.5	74.8	59.0	53.5	75.0	68.0	62.0	62.0
November	74.0	68.8	61.0	60.8	75.3	65.0	61.3	69.0
December	58.8	61.2	52.7	59.0	70.8	61.3	61.3	60.5
January	71.5	68.0	55.3	59.7	70.3	59.5	66.5	61.2
February	73.2	64.8	60.7	59.0	74.0	63.0	60.3	60.8
March	72.0	65.8	59.0	57.5	73.5	57.0	60.3	61.1
No. of Services	7,433	3,139	4,450	2,850	5,567	2,395	4,940	2,518
Average	70.5	67.2	59.1	58.3	73.2	62.3	62.0	62.4

[a] Based on 60- to 90-day nonreturns for all services.
[b] Number of bulls in each group.

No consistent difference due to exercise was found for this 6-month study. These data confirm the findings of Lepard and associates *(4)* in their 16-week trials.

Exercise did not cause any significant differences in nonreturns of high fertility Holstein sires but did seem to have a significant favorable effect in high fertility Guernsey sires. Exercise caused no significant favorable effects in low fertility bulls of either breed.

Highly significant differences were found in the average nonreturns between months and between breeds in the high fertility bulls; however, this was not true for the low fertility bulls. There were no significant differences in the average nonreturns between months and between breeds when fertility levels were combined. Highly significant differences were found for individual bulls during the experimental study.

An analysis of variance showed no significant differences between the nonreturn percentages for the 6 months that the bulls were exercised, the 6 months prior to the experimental period, or the corresponding 6 months 1 year before the exercise. This was also true with the nonexercised bulls. This confirms our earlier conclusions that exercise did not appear to have any significant effects upon the percentage of nonreturns.

Libido and general activity. This study showed that libido was not affected by forced exercise, which is in disagreement with the work of Woodward *(8)*, who reported that forced exercise greatly decreased the interval of time required to obtain a semen sample. Body weights of the exercised and nonexercised bulls showed no significant differences. No marked differences were noted between the two groups in their appetites and general well being.

SUMMARY

One group of dairy bulls (eight Holsteins and eight Guernseys) were forced-exercised on a mechanical exerciser daily, except Sundays, for 6 months while another comparable group received no exercise other than movements in their stalls. Each of these groups was divided into high and low fertility subgroups within breeds and assigned at random to treatment groups.

Data from the 853 semen ejaculates, 453 from the exercised and 400 from the nonexercised bulls, showed that exercise did not significantly favorably influence the semen characteristics studied either within or between breeds and either within or between fertility level subgroups. Breeding records (33,292), 17,872 for the exercised bulls and 15,420 for the nonexercised bulls, showed that exercise did not significantly favor a higher over-all percentage of nonreturns. The 60- to 90-day nonreturn rate for the exercised bulls was 63.8% and for the nonexercised bulls 65.0%. Further statistical analyses did reveal, however, that even though exercise did not result in any significant differences in the nonreturns of high fertility Holstein sires and in the low fertility Holstein and Guernsey sires, it did appear to improve the percentage nonreturns in high fertility Guernsey sires.

Libido was not significantly affected by exercise. The appetites and general well-being of the exercised bulls were not markedly affected.

REFERENCES

(1) BARTLETT, J. W., AND PERRY, E. J. Lessons Learned from Eighteen Months Experience with Cooperative Artificial Breeding of Dairy Cattle in New Jersey. *Proc. Am. Soc. Animal Production*, p. 243. 1939.

(2) GRADWOHL, R. B. H. *Clinical Laboratory Methods and Diagnosis.* Vol. I. p. 983. C. D. Mosby Company, St. Louis, Mo. 1943.

(3) HAMILTON, J. B., AND SYMINGTON, E. L. Management of the Dairy Bull. *Can. J. Comp. Med.*, 3: 337. 1939.

(4) LEPARD, O. L., SHUART, C. E., AND FOSTER, A. The Effect of Exercise on the Amount and Quality of a Dairy Bull's Semen. *J. Dairy Sci.*, 24: 509. 1941.

(5) SALISBURY, G. W., WILLETT, E. L., AND SELIGMAN, J. The Effect of the Method of Making Semen Smears upon the Number of Morphologically Abnormal Spermatozoa. *J. Animal Sci.*, 1: 199. 1942.

(6) SNEDECOR, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames. 1946.

(7) WEATHERBY, E. J., REECE, R. P., AND BARTLETT, J. W. The Ability of Dairy Bulls to Withstand Regular Service for Artificial Insemination During One Year. *Proc. Am. Soc. Animal Production*, p. 224. 1940.

(8) WOODWARD, E. G. The Effect of Exercise upon the Vitality and Breeding Ability of Bulls. Wash. Agr. Expt. Sta., *Bull.* 158. 1920.

THE MAMMARY ELIMINATION OF RADIOIODINE[1]

W. E. WRIGHT, J. E. CHRISTIAN, AND F. N. ANDREWS
School of Pharmacy and the Department of Animal Husbandry, Purdue University, Lafayette, Indiana

The controlled feeding of thyroactive iodinated protein to dairy cattle for purposes of increasing milk and butterfat production has been practiced by some dairymen during the past decade *(2)*. No serious abnormalities have been found in the milk from such treated cattle, and certain other experiments have failed to show that the ingestion of their milk is injurious to animals receiving it. Reineke and Turner *(8)* detected no rise in metabolic rate when milk from thyroprotein-stimulated cattle was fed to guinea pigs, and Robertson *(9)* obtained similar results upon feeding the same type of milk to humans. Courrier *et al. (4)* found only an insignificant amount of inorganic iodine as iodide in the milk of thyroprotein-treated rabbits. On the other hand, Campbell *et al. (3)* detected relatively high levels of radioactive iodine in the milk of ewes receiving doses of I^{131}-labeled iodocasein. Distribution studies carried out on the radioiodine in the ewes' milk with *n*-butanol and Blau's reagent indicated that 30% of the activity could be found in the thyroxine fraction and 50% in the diiodotyrosine-inorganic iodine fraction. Rugh *(10)* found that a sizable portion of radioiodine administered to lactating mice is transmitted to nursing litters via the milk, and Nurnberger and Lipscomb *(7)* found a similar situation to exist in humans. This study was undertaken to determine the nature and amount of any iodine compounds eliminated via the mammary gland after oral administration of I^{131}-labeled iodocasein or sodium iodide.

METHODS AND MATERIALS

I^{131}-labeled iodocasein[2] was prepared by the method of Hamilton *et al. (5)*. Purification by isoelectric precipitation and two buffer washes yielded a product free from sodium iodide. This was shown by chromatographing a solution of the purified iodocasein on paper and noting at the origin the presence of only the single peak of radioactivity due to iodocasein itself.

The chromatography technique used was similar to that employed by Taurog *et al. (12)* for the detection of thyroid constituents. The two solvent combinations used throughout this work were *(a)* collidine and water (125:44) in an atmosphere made ammoniacal by a beaker of 28% ammonium hydroxide, and *(b)* *n*-butanol, acetic acid, and water (200:30:75). In the rabbit experiment a butanol extract of the milk was concentrated and chromatographed. Subsequent experi-

Received for publication July 13, 1954.

[1] A contribution from the Bio-Nucleonics Laboratory of the School of Pharmacy and the Department of Animal Husbandry, Purdue University, as Journal Paper No. 777.

[2] The iodine[131] used in this research was obtained on allocation from the U. S. Atomic Energy Commission, Oak Ridge, Tenn.

mentation, however, revealed that monoiodotyrosine, diiodotyrosine, thyroxine, and sodium iodide could be separated chromatographically from skimmilk itself without a prior extraction step; therefore, all chromatography in the goat experimentation was carried out on skimmilk directly without the butanol extraction employed in the rabbit experiment. The positions of radioactive compounds on the chromatograms were detected by common radioautographic techniques with X-ray film.

Liquid radioactive samples were prepared for assay by pipetting the desired volume of material (usually 10μl) onto the exact center of an aluminum sample pan having a single layer of filter paper in the bottom. The I^{131} in the air-dried samples was assayed with an end window G-M tube having a mica window of less than 2 mg. per square centimeter thickness.[3]

Radioactive samples prepared for dosage were standardized by counting an aliquot volume of the material diluted with either goat's milk or cow's milk. This eliminated the necessity of making corrections for self-absorption of the sample when the I^{131} levels of milk samples were compared to the original dose. All sample values were corrected for decay, background, and coincidence when necessary.

EXPERIMENTAL PROCEDURE

Administration of I^{131}-labeled iodocasein to a lactating rabbit. As a preliminary experiment, 0.18 g. of radioactive iodocasein prepared from a starting I^{131} activity of 5.4 mc. was administered in solution by stomach tube to a lactating rabbit with a 10-day-old litter. The rabbit was milked 6 hours post-administration with the milking technique of Bergman and Turner *(1)*. The skimmilk portion was diluted with an equal part of water, acidified to pH 3, and extracted three times with equal volumes of *n*-butanol, which is reported to extract monoiodotyrosine, diiodotyrosine, thyroxine, and inorganic iodide from a variety of solutions *(6, 11)*. This butanol extract was concentrated to one-tenth volume and chromatographed in the previously described collidine-water-ammonia solvent both with and without added carriers of diiodotyrosine, thyroxine, and labeled sodium iodide.

Radioautography of the resulting chromatograms demonstrated the presence of radioactive compounds at the positions normally occupied by monoiodotyrosine, diiodotyrosine, and iodide. No activity at the thyroxine position could be detected. (See Figure 1.) A fourth peak at Rf 0.65 comprising the majority of the radioactivity of the strip was also observed and for a time caused confusion in the interpretation of the results; however, this fourth constituent was finally identified as an artifact of unknown composition arising from the concentration of iodide in acid butanol solution. When a butanol extract of a simple aqueous labeled sodium iodide solution was concentrated and chromatographed, the artifact appeared, indicating that it was a product arising from the concentration step and was not a component of the milk itself.

[3] A Tracerlab T.G.C.-1 G-M tube was mounted in a Tracerlab SC-10A Radioassay Sample Holder and attached to a Nuclear Instrument and Chemical Corp. No. 165 Scaling Unit.

Administration of I^{131}-labeled iodocasein to lactating goats. As experimental animals, lactating goats offered the advantages of constant lactation, uncomplicated by nursing young, and the opportunity of collecting and processing all of the milk produced by a specific animal.

Experiments were carried out in which two Nubian lactating goats received oral doses of I^{131}-labeled iodocasein in a dosage ratio of 1.5 g. of iodocasein per 100 lb. of body weight. The 1.7 g. of iodocasein administered to Goat A was synthesized from I^{131} having an initial activity of 23.7 mc. and, when dissolved in 40 ml. of 1% sodium bicarbonate solution for administration, assayed 3.02×10^7 c.p.m. per milliliter by the technique mentioned previously. Goat B received 80 ml. of a similar solution containing 1.6 g. of iodocasein synthesized from 32.5 mc. of I^{131} and assaying 2.1×10^7 c.p.m. per milliliter.

The goats were milked every 12 hours post-administration, and chromatograms of 5-μl. skimmilk samples of the first ten collections were prepared in the two different solvent combinations described under Methods and Materials. A reproduction of a representative radioautograph of a collidine solvent chromatogram can be examined in Figure 1. The only exposed spot that can be seen is

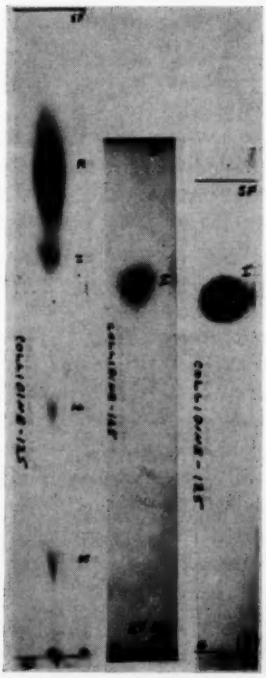


FIG. 1. Radioautographs of chromatograms. 1. Butanol extract of skimmilk from a labeled iodocasein-treated rabbit. 2. Skimmilk from a labeled iodocasein-treated goat. 3. Skimmilk from a label sodium iodide-treated goat. O = origin, DI = diiodotyrosine, M = monoiodotyrosine, I = iodide, A = artifact, SF = solvent front.

at the Rf position normally taken by iodide. No spots due to monoiodotyrosine, diiodotyrosine, or thyroxine could be detected on chromatograms run in either of the two solvent combinations used.

The per cent of administered iodocasein I^{131} eliminated by the mammary gland in each 12-hour milking period was calculated by counting 10-μl. aliquot samples of whole milk from each collection and multiplying the activity of the aliquot by the total volume of milk collected in that specific period. The curves in Figure 2 were prepared from the values for the per cent of administered iodine

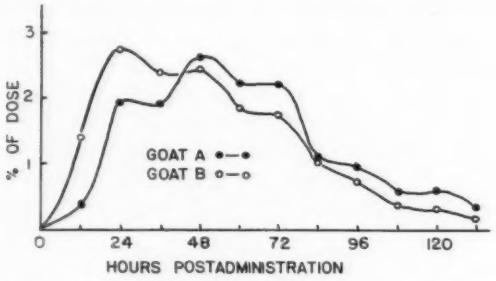


FIG. 2. Rate of appearance of I^{131} in the milk following labeled iodocasein administration to lactating goats.

eliminated in each milking period and show the rate at which I^{131} appeared in the milk. The sum of the I^{131} activity excreted in the milk during the experimental period was determined and expressed as a per cent of the total I^{131} activity administred. These values were found to be 15.1% and 15.8% (average, 15.5%) for Goats A and B, respectively.

Administration of I^{131}-labeled sodium iodide to lactating goats. Four experiments were carried out involving the administration of I^{131}-labeled sodium iodide to lactating goats. In the first three experiments, the dose of sodium iodide administered was intended to furnish to the animal the same weight of iodine that the animal would have received if iodocasein had been administered. The following oral doses were administered: Experiment 1, 20 ml. of a solution containing 72 mg. of sodium iodide and having an I^{131} activity of 3.61×10^7 c.p.m. per milliliter; Experiment 2, 20 ml. of a solution containing 189 mg. of sodium iodide and having an I^{131} activity of 5.23×10^7 c.p.m. per milliliter; Experiment 3, 40 ml. of a solution containing 240 mg. of sodium iodide and having an activity of 4.31×10^7 c.p.m. per milliliter. In the fourth experiment, 5 ml. of a solution of carrier-free sodium iodide^{131} counting 4.31×10^7 c.p.m. per milliliter was administered. Techniques of milk sampling, chromatography, and radioassay were the same as those explained previously in the iodocasein experiments.

The rate of mammary elimination of I^{131} in each experiment, expressed as per cent of administered dose eliminated in each successive 12-hour milking period, is represented graphically in Figure 3. In the first three experiments, each covering a span of about 7 days, 53.5%, 45.3%, and 51.3%, respectively,

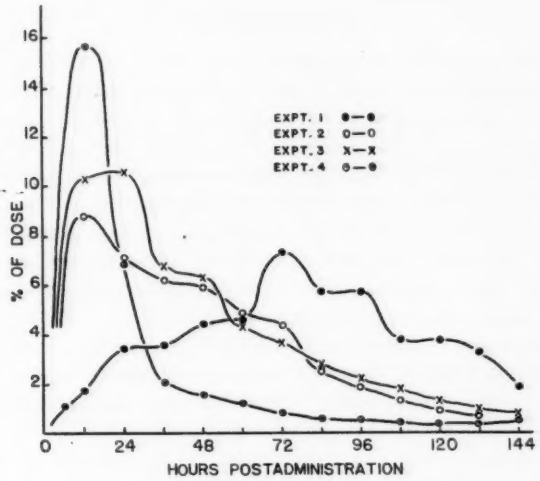


FIG. 3. Rate of appearance of I^{133} in the milk following sodium iodide^{131} administration to lactating goats.

of the administered doses of sodium iodide were eliminated by the mammary route. This represents an average mammary elimination of 50.0%. In the fourth experiment 31.2% of the administered carrier-free I^{131} was found in the milk.

The animal used in Experiment 2 was placed in a large metabolism cage designed for the separate collection of urine and feces in order to determine approximately the I^{131} eliminated by these routes. Of the administered dose of I^{131}, 40.7% was eliminated in the urine and 2.3% was detected in the feces over a period of 180 hours, but 12.7% could not be accounted for by the technique; however, since the animal was still excreting small amounts of iodine at the end of the experimental period, at least part of the remaining iodine was still in the animal with a considerable amount still bound by the thyroid. Undoubtedly a small portion of iodine excreted in the urine was unrecovered from the floor of the cage itself, and other small fractions were lost in experimental manipulations.

The only radioactive compound detected in the milk by chromatography and radioautography was iodide. A representative collidine solvent chromatogram demonstrating this fact is shown in Figure 1.

It was to be expected that a relatively large dose of sodium iodide administered to an animal would be eliminated for the most part unchanged, and such was the case in the administration of iodide to goats. However, the fact that more than half of the iodide dose was eliminated in the milk would lead to the belief that in some animals functioning mammary glands are as efficient organs of elimination for certain compounds as are the kidneys. The milk and urine produced in the experimental period were nearly equal in volume.

SUMMARY

The oral administration of I^{131}-labeled iodocasein to a lactating rabbit resulted in the identification of monoiodotyrosine, diiodotyrosine, and iodide in the milk. The oral administration of single doses to lactating goats resulted in the mammary elimination of an average of 15.5% of the administered iodine as iodide. Oral administration to lactating goats resulted in the mammary elimination of an average of 50% of the administered radioactive iodine as iodide. In a single experiment, the levels of elimination of orally administered sodium $iodide^{131}$ in the milk, urine, and feces were 45.3%, 40.7%, and 2.3%, respectively. In a single experiment 31.2% of an oral dose of carrier-free sodium $iodide^{131}$ was eliminated in the milk of a lactating goat as iodide.

A chromatographic-radioautographic technique capable of separating monoiodotyrosine, diiodotyrosine, thyroxine, and sodium iodide from skimmilk failed to detect the presence of any iodine compounds other than iodide in the milk of goats treated with I^{131}-labeled iodocasein or sodium iodide.

REFERENCES

(1) Bergman, A. J., and Turner, C. W. The Composition of Rabbit Milk Stimulated by the Lactogenic Hormone. *J. Biol. Chem.*, **120**: 21. 1937.

(2) Blaxter, K. L. Some Effects of Thyroxine and Iodinated Casein on Dairy Cows, and Their Practical Significance. *Vitamins and Hormones*, **10**: 217. 1952.

(3) Campbell, D. J., Andrews, F. N., and Christian, J. E. A Study of the Absorption Characteristics of $Iodine^{131}$ Labeled Iodinated Casein in Some Mammals. *Endocrinology*, **47**: 242. 1950.

(4) Courrier, R., Roche, J., Deltour, G. H., Morois, M., Michel, R., and Morel, F. Sur l'excretion mammaire d'iode radioactif après administration d'iodures ou d'iodocaseine marques. *Compt. rend. soc. biol.*, **143**: 599. 1949.

(5) Hamilton, C. F., Power, M. H., and Albert, A. Preparation of Radioactive Iodocasein. *J. Biol. Chem.*, **178**: 213. 1949.

(6) Leland, J. P., and Foster, G. L. A Method for the Determination of Thyroxine in the Thyroid. *J. Biol. Chem.*, **95**: 165. 1932.

(7) Nurnberger, C. E., and Lipscomb, A. Transmission of Radioiodine (I^{131}) to Infants Through Human Maternal Milk. *J. Am. Med. Assoc.*, **150**: 1398. 1952.

(8) Reineke, E. P., and Turner, C. W. Non-Permeability of the Mammary Gland to Thyroid Hormone. *J. Dairy Sci.*, **27**: 793. 1944.

(9) Robertson, J. D. The Preparation and Biological Effects of Iodinated Proteins. 5. The Effect on Basal Metabolism of Milk from Cows Fed with Iodinated Protein. *J. Endocrinol.*, **4**: 300. 1945.

(10) Rugh, R. The Mouse Thyroid and Radioactive Iodine (I^{131}). *J. Morphol.*, **89**: 323. 1951.

(11) Taurog, A., and Chaikoff, I. L. The Nature of the Circulating Thyroid Hormone. *J. Biol. Chem.*, **176**: 639. 1948.

(12) Taurog, A., Tong, W., and Chaikoff, I. L. The Monoiodotyrosine Content of the Thyroid Gland. *J. Biol. Chem.*, **184**: 83. 1950.

MILK LIPASE[1,2]

T. W. ALBRECHT AND H. O. JAYNES
Department of Dairying
Tennessee Agricultural Experiment Station, Knoxville

The objective of this work was to establish the presence or absence of a lipolytic enzyme in raw milk which was active within the pH range of 5.0 to 5.5 so that additional information might be gained about Cheddar cheese ripening.

Lipolysis has been recognized as a factor causing a desirable or an undesirable change in dairy products. Fouts and Weaver *(3)* and Weaver *(21)* have shown that from 10.4 to 14.3% of the cows they studied produced milk which became rancid in approximately 15 hours. Hansen and Theophilus *(7)* and Jack *et al. (10)* found the enzyme lipase responsible for rancid or other less easily defined off flavor in butter. Hlynka and Hood *(9)* showed that lipolytic activity causes a rancid flavor to develop in raw milk Cheddar cheese. Evidence has been presented by Lane and Hammer *(13)* to show that controlled amounts of the enzyme added to pasteurized milk used in the making of Cheddar cheese can influence the development of a desirable flavor in the finished product.

There is some question in the literature as to whether the lipase in milk is a single enzyme or a mixture of several enzymes. Herrington and Krukovsky *(8)* postulated at least two enzymes on the basis of formaldehyde sensitivity. This was substantiated by the work of Peterson *et al. (15)* and Roahen and Sommer *(18)*. The latter authors also showed the possibility of two enzymes by the fact that a part of the lipase activity of milk disappears after 5 to 10 hours of holding. The lipase in milk has been found to hydrolyze a number of different fats and fatty acid esters. Gould *(5)* showed that milk lipase will hydrolyze butterfat, cottonseed oil, margarine, hydrogenated cooking fat, corn oil, linseed oil, olive oil, castor oil, coconut oil, and lard. Kelly *(11, 12)* found milk lipase able to hydrolyze tributyrin, tricaproin, triacetin, tripalmitin, butter oil, ethyl oleate, and diacetin. The rate of hydrolysis of the pure fats varied inversely with the length of the fatty acid chains. Reder *(17)*, Mattick and Kay *(14)*, Roahen and Sommer *(18)*, and Peterson *et al. (15)* also have demonstrated the ability of the milk lipase to hydrolyze tributyrin. Greenbank *(6)* has shown that the lipase in milk can hydrolyze α-naphthyl esters of fatty acids.

Roahen and Sommer *(18)* have found an optimum lipase activity in sugar saturated cream to be at pH 8.4-8.6, and Gould *(5)* demonstrated an optimum at a pH 8.0-9.0 for the hydrolysis of butterfat homogenized into rennet whey from raw milk. Mattick and Kay *(14)*, using a tributyrin substrate, found an optimum

Received for publication July 15, 1954.

[1] This work was supported in part by funds from Marschall Dairy Laboratories, Inc., Madison, Wis.

[2] Data submitted by H. O. Jaynes in partial fulfillment of the requirements for a Master of Science degree.

pH for lipase action at 8.2-8.7. Peterson *et al. (15)* demonstrated an optimum at pH 8.5 for the hydrolysis of tributyrin by milk lipase but found a very little or no activity in the range of pH 5.0-5.5.

EXPERIMENTAL PROCEDURE

The milk used in the investigation was of grade A quality as received by the University of Tennessee Creamery. In a number of cases the fat was separated from the milk by use of an International Centrifuge, size 1, type S. B. at 2,000 r.p.m. for 10 minutes. With this procedure it was difficult to obtain a skimmilk with as low a fat content as that produced by the De Laval cold milk separator used in the University of Tennessee Creamery, and therefore most of the skimmilk was obtained by the use of the De Laval machine.

Tributyrin, tricaproin, tricaprylin, and ethyl butyrate obtained from the Eastman Kodak Company were used as substrates. Commercial rennet extract was obtained from Marschall Dairy Laboratories, Inc., for the experiments with rennet whey.

In this investigation the method of Peterson *et al. (15)* for the estimation of lipase activity in raw skimmilk was used with the following modifications. The incubation time was extended to 90 minutes. Adjustments of pH were made with 85% lactic acid. Incubations were made in a Precision Scientific Company Model C-2 constant temperature water bath with a rated accuracy of 0.5° C., and pH measurements were made with a glass electrode, using a Beckman Model H pH meter. In all tests made at or below a pH of normal milk, no buffer was used.

The effect of pH on lipolysis. Since milk contains considerable buffering ability, it was deemed unnecessary to add additional buffers when making pH adjustments in the range of 6.6 to 5.0. Unless otherwise stated, the adjustments of pH were made with 85% lactic acid, as it is the major acid produced by culture bacteria in the manufacture of Cheddar cheese. During the first part of the work, pH adjustments were made by adding the acid to the milks, placing the acidified skimmilk in a refrigerator for a period of 30 minutes, and readjusting a second time. It was found, however, that this period of cool-aging was not long enough to maintain the pH of the acidified milk at a constant value. The pH of the acidified milks rose as much as 0.3 of a pH unit in 24 hours. Therefore, in order to obtain pH values which would remain constant, pH adjustments were made with the acid, the adjusted skimmilks were stored for 18 to 24 hours at 4° C., then warmed to 40° C., and a second pH adjustment was made with the addition of the same acid. After the second adjustment was made, the pH remained constant during the next 24 hours of cool-aging.

The effect of the lipolysis of tributyrin within a pH range of 5.0 to 8.5 is shown in Figure 1. The intermediate points between the pH of the normal skimmilk and the pH of 8.5 were not determined, consequently the broken line. The hydrolysis at the pH of 8.5 was an average of eight trials and was submitted only to show the relative activity of the peaks. In the pH range below 6.6 two activity peaks were observed, one at pH 6.3 and the other at 5.4. This portion

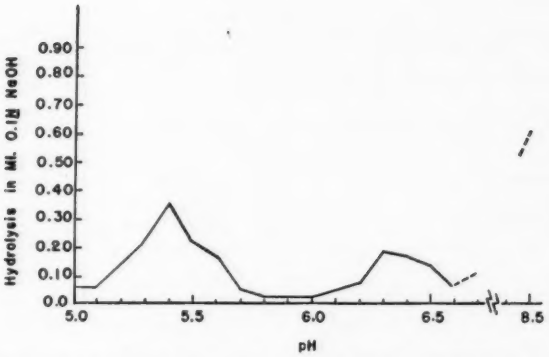


FIG. 1. Effect of pH on the rate of tributyrin hydrolysis by milk lipases.

of the curve is an average of 10 trials. Lipase activity was evident through the pH range of 5.0 and 5.7 and pH 6.0 and 6.6. Very slight activity, and sometimes none at all, was observed in the range of pH 5.7 to 6.0.

Effect of some cations and anions on the lipolytic activity. Previous investigations *(1, 15, 20)* have shown that certain salts have a marked effect on the activity of milk lipases. In this experiment the salts were added to raw skimmilk as 1.0 *M* aqueous solutions in such quantities as to give 0.1 *M* added concentration in the milk. After adding the salt to the milk, the pH was adjusted to 6.3 and 5.4 with 85% lactic acid, the mixture was stored for 18-24 hours at 4° C., the pH was readjusted to 6.3 and 5.4, and the lipase activity tests were conducted. Chloride and lactate salts of calcium, magnesium, sodium, and potassium were used. The results are shown in Table 1. $CaCl_2$ lowered the

TABLE 1

Effects of certain salts on lipolysis of tributyrin by milk lipase

Salt used	Molar concentration	Titration differences[a] pH 6.3	Titration differences[a] pH 5.4
		(Experiment I)	
Control	—	0.33	0.385
$CaCl_2$	0.1	0.12	0.22
$MgCl_2$	0.1	0.27	0.56
NaCl	0.1	0.06	0.07
KCl	0.1	0.145	0.27
		(Experiment II)	
Control[b]	—	0.19	0.35
Ca lactate	0.1	0.17	0.34
Mg lactate	0.1	0.17	0.39
Na lactate	0.1	0.10	0.15
K lactate	0.1	0.13	0.21

[a] Titration differences represent tributyrin hydrolysis by lipase. They are in terms of ml. 0.1 *N* acid released in 90 minutes by 5 ml. of milk held at 40° C. The term "titration difference" is used throughout in this sense.

[b] Different milk used.

TABLE 2

Effects of certain acids on hydrolysis of tributyrin by milk lipase

Acid used	Titration differences pH 6.3	Titration differences pH 5.4
Lactic	0.33	0.47
Citric	0.18	0.31
Phosphoric	0.13	0.25
Acetic	0.01	0.02
Hydrochloric	0.005	0.01
Sulfuric	0.01	0.015

lipase activity of both the pH 6.3 and 5.4 peaks. $MgCl_2$ produced no significant change of the pH 6.3 peak but appeared to be stimulatory at the pH 5.4 peak. NaCl produced a marked inhibition at both pH peaks. KCl had considerable inhibiting action but less than $CaCl_2$ or NaCl. Similar results were obtained when the lactate salts were added to the milk, with the exception of the calcium salt. Calcium lactate was not significantly inhibitory at either of the pH peaks.

Since the lipase activity optima at pH 6.3 and 5.4 were established with lactic acid to make the pH adjustments, a series of experiments was conducted to study the effect of other acids at the same pH optima. Acids used were concentrated sulfuric, hydrochloric, glacial acetic, 85% phosphoric, and a saturated solution of citric acid. The averages of four trials appear in Table 2. Acetic, hydrochloric, and sulfuric acids almost completely inhibited the lipase activity at pH 6.3 and 5.4 as compared to the milks acidified with lactic acid. Citric and phosphoric acids also were inhibitory, but not nearly to the extent of the other acids.

Comparison of substrates. Gould *(5)* and Kelly *(11, 12)* found a wide range of substrates acceptable for the pH 8.5 lipase enzyme in raw milk, and the rate of lipase hydrolysis was inversely proportional to the length of the fatty acid. In this study, tributyrin, tricaproin, and tricaprylin were compared, as shown in Figure 2. The pH curves are similar in shape with the exception of tricaproin,

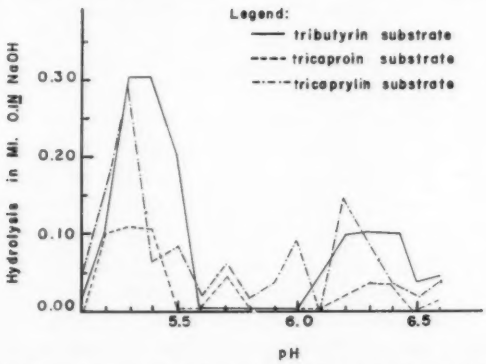


FIG. 2. Effect of pH on the rate of hydrolysis of tributyrin, tricaproin, and tricaprylin by the milk lipases.

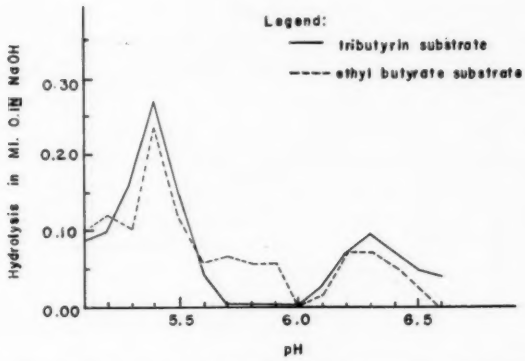


FIG. 3. Effect of pH on the rate of hydrolysis of tributyrin and ethyl butyrate by milk lipases.

which was hydrolyzed between the pH of 5.7 and 6.0. Figure 3 compares the hydrolysis of tributyrin and ethyl butyrate. The ethyl butyrate was hydrolyzed in the same pH range as that observed for tributyrin with maximum hydrolysis at pH 6.3 and 5.4. In addition, within the pH range of 5.6 and 6.0, the ethyl butyrate was also hydrolyzed. This work was repeated three times, and the pH range of hydrolysis was always the same.

The effect of adding formaldehyde. There is some disagreement in the literature concerning the effect of formaldehyde on milk lipase. The work of Roahen and Sommer *(18)*, Herrington and Krukovsky *(8)*, and Peterson *et al. (15)* showed that formaldehyde greatly reduces lipase activity in milk. Using the sensitivity of milk lipase toward formaldehyde, Herrington and Krukovsky postulated two lipases in milk, one sensitive to formaldehyde and one not. On the other hand, Gould *(4)* and Tarassuk and Richardson *(19)* maintained that the addition of formaldehyde to milk does not inhibit lipase activity. An experiment was set up to determine the effect of adding formaldehyde to milk upon the lipase and esterase activity between a pH of 5.1 and 6.6. Formaldehyde (37%) was added to raw skimmilk at the rate of 2 g. per liter. A portion of the same milk was retained with no formaldehyde added. Adjustments in pH were made on the two samples, and the lipase and esterase activities were determined. Figure 4 shows the result of this experiment. The addition of the formaldehyde reduced the total enzyme activity for both tributyrin and ethyl butyrate. In the range of pH 5.7 and 6.0, the hydrolysis of ethyl butyrate was not evident. Therefore, an esterase active in this pH range was inactivated by the addition of the formaldehyde to the skimmilk.

Lipase activity of casein and whey. Dorner and Widmer *(2)* attempted in their study of homogenization effects on rancidity to ascertain which portion of the milk carried the lipase. Their conclusion was that it accompanied the casein or that an activator for the enzyme accompanied the casein. Mattick and Kay *(14)* believed the lipase was present in the aqueous rather than the fatty

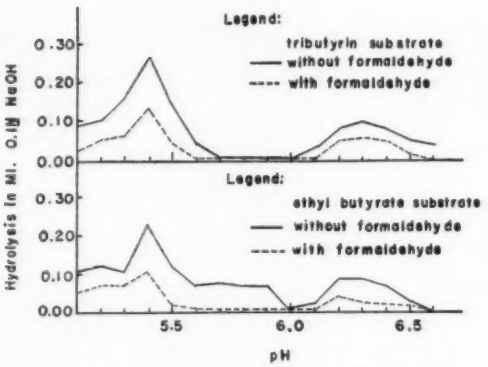


FIG. 4. Effect of formaldehyde upon the rate of hydrolysis of tributyrin and ethyl butyrate by milk lipases.

portion of the milk. Using milk made from 40% cream and skimmilk when one or the other had been pasteurized, Gould *(4)* found that most of the enzyme was in the skimmilk. He also compared the lipolytic activity of skimmilk and rennet whey on melted butter oil, finding again that most of the enzyme was in the skimmilk. A series of experiments was carried out to determine whether the lipases active in milk at pH 6.3 and 5.4 were associated with the casein or with the whey proteins of skimmilk.

In the first experiment a sample of raw skimmilk was adjusted to pH 6.3 with 85% lactic acid and precipitated with concentrated rennet extract (5 ml. added per 1,000 ml. skimmilk) at 30° C. A portion of the original milk was retained. Portions of the milk and rennet whey, filtered through a fluted filter paper and unfiltered, were adjusted to pH 6.3 and 5.4 with 85% lactic acid and stored overnight at 4° C., the pH was readjusted, and the lipase activity tests were carried out. The results are shown in Table 3. Samples of skimmilk hydro-

TABLE 3

Hydrolysis of tributyrin and ethyl butyrate by skimmilk, filtered rennet whey, and unfiltered rennet whey

		Adjusted	After storage	Titration
Sample	Substrate	pH	pH	differences
Raw skimmilk	Tributyrin	6.3	6.38	0.07
Raw skimmilk	Ethyl butyrate	6.3		0.04
Raw skimmilk	Tributyrin	5.4	5.61	0.31
Raw skimmilk	Ethyl butyrate	5.4		0.31
Filtered whey	Tributyrin	6.3	6.31	0.00
Filtered whey	Ethyl butyrate	6.3		0.00
Filtered whey	Tributyrin	5.4	5.42	0.00
Filtered whey	Ethyl butyrate	5.4		0.00
Unfiltered whey	Tributyrin	6.3	6.30	0.00
Unfiltered whey	Ethyl butyrate	6.3		0.00
Unfiltered whey	Tributyrin	5.4	5.40	0.00
Unfiltered whey	Ethyl butyrate	5.4		0.00

lyzed tributyrin and ethyl butyrate, but the whey did not. No attempt was made to evaluate the lipase in the rennet curd because of the retarding action of sodium and potassium ions and the inability of the calcium or magnesium ions to dissolve the rennet curd.

The experiment was repeated with acid coagulation used in place of the rennet. A sample of raw skimmilk was divided into two parts. One portion served as a control and the other portion was precipitated by adjusting the pH to 4.7 with 85% lactic acid at 25° C. The acid whey from the coagulated milk showed a pH of 4.62 after draining. A part of this whey was filtered through a fluted filter paper and another part retained without filtering. The pH of the skimmilk was adjusted to pH 6.3 and 5.4 with 85% lactic acid. Portions of the filtered and unfiltered whey were adjusted to pH 6.3 and 5.4 with a heavy suspension of $Ca(OH)_2$. The curd was resuspended in enough distilled water containing $Ca(OH)_2$ to give approximately the concentration of resuspended curd that originally existed in the milk. Portions of the curd suspensions were adjusted to pH 6.3 and 5.4 with 85% lactic acid. All samples were stored overnight at 4° C., and lipase activities were carried out the succeeding day after the usual readjustments of pH. The results are shown in Table 4. The resuspended curd showed lipase and esterase activity comparable to that of the original skimmilk. No activity was shown by the whey samples.

TABLE 4

Hydrolysis of tributyrin and ethyl butyrate by skimmilk, lactic acid whey, and resuspended lactic acid curd

Sample	Substrate	Adjusted pH	After storage pH	Titration differences
Raw skimmilk	Tributyrin	6.3	6.36	0.07
	Ethyl butyrate	6.3		0.06
	Tributyrin	5.4	5.78	0.11
	Ethyl butyrate	5.4		0.10
Acid whey	Tributyrin	6.3	6.33	0.00
	Ethyl butyrate	6.3		0.00
	Tributyrin	5.4	5.70	0.00
	Ethyl butyrate	5.4		0.00
Resuspended acid curd	Tributyrin	6.3	6.40	0.06
	Ethyl butyrate	6.3		0.06
	Tributyrin	5.4	5.52	0.10
	Ethyl butyrate	5.4		0.10

The effect of temperature on lipolysis. Throughout the experimental work an incubation temperature of 40° C. was used. This was the temperature recommended by Peterson *et al. (15)* for the hydrolysis of tributyrin at pH 8.5. An experiment varying incubation temperatures with the lipases active at pH 6.3 and 5.4 was conducted. Tributyrin was used as the substrate. The results of this experiment are shown in Figure 5. Under the conditions of the experiment, the pH 6.3 system had a maximum activity at approximately 37° C. and the pH 5.4 system at approximately 40° C.

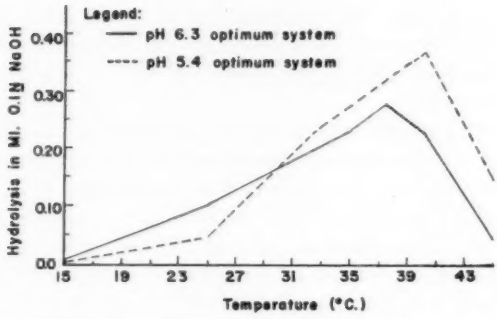


FIG. 5. Effect of temperature on the rate of hydrolysis of tributyrin by the milk lipases.

DISCUSSION

Many esterases, which include the lipases, have a low degree of substrate specificity and consequently are capable of hydrolyzing the simple esters, as well as fats. In this study, two general systems were observed with optima at pH 5.4 and 6.3 that could hydrolyze both tributyrin and ethyl butyrate. Upon the addition of formaldehyde to the raw skimmilk, the enzymatic activity of both systems was reduced about 50%. Herrington and Krukovsky *(8)* postulated two lipases in milk based upon the behavior of the enzymes toward formaldehyde. In Figure 6, a comparison of the rates of hydrolysis for the enzymes inhibited by the addition of formaldehyde and those not inhibited is shown. In the pH ranges of the 5.4 and 6.3 optima systems, the formaldehyde-sensitive enzyme was able to hydrolyze both tributyrin and ethyl butyrate equally well. But with

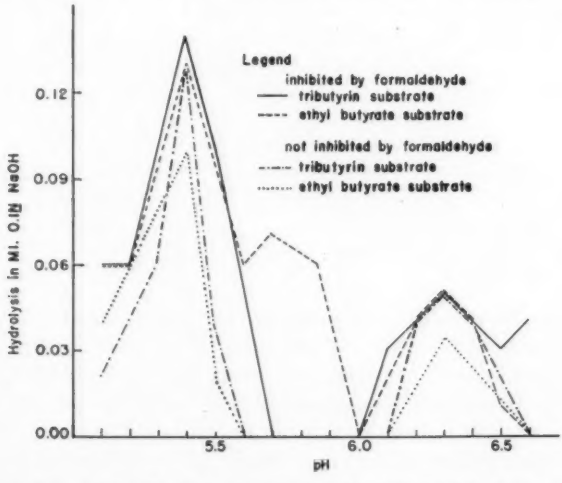


FIG. 6. Comparison of the lipolytic enzymes in skimmilk inhibited and not inhibited by formaldehyde when tributyrin or ethyl butyrate was used as a substrate.

the enzymes not inhibited by the formaldehyde, the ethyl butyrate was hydrolyzed about 25% slower. Based upon the formaldehyde sensitivity and specificity of the enzyme for the two substrates, this work tends to show two lipase enzymes within each of the general systems. In the pH range of 5.7 to 6.0, ethyl butyrate was hydrolyzed but tributyrin was not, and when formaldehyde was added to the skimmilk the hydrolysis of ethyl butyrate was completely inhibited. In this zone the enzyme appears to be an esterase, specific for ethyl butyrate and not for tributyrin.

The suggestion has been made by Peterson *et al. (16)* that the origin of lipolytic enzymes in raw milk Cheddar cheese was the bacteria in the milk and the rennet extract but not the milk lipases. They based their conclusion upon the rapid reduction in the activity of the pH 8.5 optimum lipase system when it was made acid and held at temperatures used in the cheesemaking operation. Within the limits of this experiment the lipases with an optimum at either pH 5.4 or 6.3 were quantitatively removed from the whey, either by lactic acid or rennet coagulation of the casein, and upon resuspending the acid curd to a similar volume of the original milk the activity was not changed. In addition, all of the samples of milk used in the study were acidified and stored at least 18 hours before the activity was measured. Therefore, a portion of the cheese lipase observed by Peterson *et al. (16)* in the raw milk Cheddar cheese and not in the pasteurized milk cheese was not totally of bacterial origin but was a natural part of the milk. The inability of previous investigators to observe the lipases at the lower pH range was very likely because of the choice of acids used to acidify the milk.

SUMMARY

Two general lipase systems have been demonstrated in raw skimmilk within the pH range of 5.0 and 6.6, with optima at pH 5.4 and 6.3. Based upon formaldehyde sensitivity and substrate specificity, four lipase enzymes have been observed, two in each general system. An esterase was observed within the pH range of 5.7 and 6.0 which was inactivated by the addition of formaldehyde. The lipase can be quantitatively removed by precipitation of the skimmilk with rennet or acid (to the isoelectric point).

REFERENCES

(1) Davies, W. L. The Inactivation of Lipase in Dairy Products by Traces of Heavy Metal Salts. *J. Dairy Research*, **3**: 254. 1932.

(2) Dorner, W., and Widmer, H. Homogenization and Milk Rancidity. *Milk Plant Monthly*, **21** (7): 50. 1932.

(3) Fouts, E. L., and Weaver, E. Observations on the Development of Rancidity in Sweet Cream and Butter. *J. Dairy Sci.*, **19**: 482. 1936.

(4) Gould, I. A. Effect of Certain Factors upon Lipolysis in Homogenized Raw Milk and Cream. *J. Dairy Sci.*, **24**: 779. 1941.

(5) Gould, I. A. Influence of pH, Type of Fat, and Pancreatic Extract upon Lipolysis in Homogenized Milk. *J. Dairy Sci.*, **25**: 869. 1942.

(6) Greenbank, G. R. A Colorimetric Determination of Lipase. *J. Dairy Sci.*, **33**: 396. 1950.

(7) HANSEN, H. C., AND THEOPHILUS, D. R. Cause of Wintery Flavor in Butter Established. Idaho Agr. Expt. Sta., *Bull.* 244. 1939.

(8) HERRINGTON, B. L., AND KRUKOVSKY, V. N. Studies of Lipase Action. I. Lipase Action in Normal Milk. *J. Dairy Sci.*, **22**: 127. 1939.

(9) HLYNKA, I., AND HOOD, E. G. Milk Lipase and Milk Flavor. *J. Dairy Sci.*, **25**: 389. 1942.

(10) JACK, E. L., TARASSUK, N. P., AND SCARAMELLA, E. L. Effect of Pasteurization Temperatures on Keeping Quality of Butter Made from Cream Containing Naturally Active Lipase. *Natl. Butter Cheese J.*, **33** (12): 16. 1942.

(11) KELLY, P. L. Milk Lipase Activity, a Method for Its Determination and Its Relation to the Estrus Cycle. *J. Dairy Sci.*, **28**: 803. 1945.

(12) KELLY, P. L. The Determination of Lipase from Milk Extracted with Acetone and Ether. *J. Dairy Sci.*, **27**: 675. 1944.

(13) LANE, C. B., AND HAMMER, B. W. Effect of Lipolytic Enzyme on the Ripening of Cheddar Cheese. *J. Dairy Sci.*, **23**: 519. 1940.

(14) MATTICK, E. C. V., AND KAY, H. D. A Lipase (Tributyrinase) of Cow's Milk. Occurrence, Method of Estimating, and Relation to Lactation Cycle. *J. Dairy Research*, **9**: 58. 1938.

(15) PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Determination of Milk Lipase. *J. Dairy Sci.*, **26**: 233. 1943.

(16) PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Lipase Activity During Making and Ripening of Cheddar Cheese. *J. Dairy Sci.*, **31**: 39. 1948.

(17) REDER, RUTH. The Chemical Composition of Normal and Rancid Jersey Milk. III. Titratable Acidity, Hydrogen Ion Concentration, and Lipase Content. *J. Dairy Sci.*, **21**: 369. 1938.

(18) ROAHEN, D. C., AND SOMMER, H. H. Lipolytic Activity in Milk and Cream. *J. Dairy Sci.*, **23**: 831. 1940.

(19) TARASSUK, N. P., AND RICHARDSON, G. A. Significance of Lipolysis in the Curd Tension and Rennet Coagulation of Milk. I. The Role of the Fat Globule Absorption Membrane. II. The Effect of the Addition of Certain Fatty Acids to Milk. *J. Dairy Sci.*, **24**: 667. 1941.

(20) TUCKEY, S. L., AND RUEHE, H. A. Relation of Salt to Bitter Flavor Development in Cheddar Cheese. *J. Dairy Sci.*, **23**: 517. 1940.

(21) WEAVER, E. Physiological Factors Affecting Milk Flavor with a Consideration of the Validity of Flavor Scores. Okla. Agr. Expt. Sta., *Bull.* 6. 1939.

A MODIFIED PROCEDURE FOR THE SEPARATION OF ACIDIC AMINO COMPOUNDS USING A SULFONATED POLYSTYRENE RESIN[1,2]

M. K. HAMDY, W. J. HARPER, AND H. H. WEISER
Departments of Dairy Technology and Bacteriology
Institute of Nutrition and Food Technology
The Ohio State University, Columbus

The recent development of high capacity and durable ion-exchange resins has stimulated much interest in the application of these resins in many divergent fields of science. Ion-exchange chromatography, which has been used successfully for the separation of many different classes of compounds, is one of the most valuable applications.

Stein and Moore *(9)*, Black *(1)*, Bryant *(2)*, Moore and Stein *(4)*, Hirs *et al. (3)*, Partridge *et al. (6)*, Tabor and Hayaiski *(10)*, and Wall *(12)* have utilized ion-exchange chromatography for the separation of amino acids on a laboratory scale. Although these methods have been valuable, their time requirements have limited their usefulness in studies in which large numbers of analyses are necessary.

In cheese ripening studies, the more acidic amino compounds, such as glutamic acid, serine, and proline, have been of particular interest. The objective of this study was limited to adapt the technique of ion-exchange chromatography for the acidic amino compounds for more rapid routine analyses of starter cultures and cheese.

EXPERIMENTAL PROCEDURE

The sulfonated polystyrene ion-exchange chromatographic method of Moore and Stein *(4)* was selected as the fundamental procedure in this investigation. Various trials were made to increase the rate of separation of amino acids by this technique.

Attempts to increase the rate of separation of the amino compounds at room temperature instead of 37° C. by shortening the column and altering the flow rate of the pH 3.42 buffer of Moore and Stein were not successful. The second approach was to alter the pH of the buffer with the view of increasing the rate of elution of the acidic amino compounds, as suggested by Moore and Stein. A slight increase in the rate of elution was achieved with pH 4.0 buffer (0.1 *M*), but at room temperature the predicted alteration in the relative position of the amino compounds reported by Moore and Stein did not occur. Furthermore, this modification was unsatisfactory, since alteration in room temperatures caused

Received for publication July 15, 1954.

[1] Funds provided in part by the Ohio State University Development Fund through the Institute of Nutrition and Food Technology, The Ohio State University, by the Keenan Fund, and by the Research and Marketing Act of 1946, through the Dairy Products Section, Utilization Research Service, USDA.

[2] Scientific article No. 10-54, Department of Dairy Technology, The Ohio State University. Ohio Agr. Expt. Sta. Journal article 38-54.

a variation in the threshold volume of the various amino acids. Attempts to minimize temperature fluctuation by use of a water-jacketed column to control the temperature at 20° C. materially altered the operation of the column and interfered with the separation of the acids.

Finally it was found that dilution of the pH 4.0 buffer to 0.06 *M* permitted a four- to five-fold increase in the flow rate with almost the same pattern of separation reported by Moore and Stein *(1)* using a buffer at pH 3.42, 0.1 *M* in a column 0.9 × 100 cm. at 37.5° C.

Variations in temperature from 20° to 30° C. did not alter the threshold volume for the elution for any of the amino compounds studied. The increase in the flow rate necessitated a 10-cm. increase in the length of the resin bed in the column for the best separation.

Initially, the composition of the buffer was controlled by adjusting it with a hydrometer to a Baumé reading of 1.05. For more accurate control, the molar concentration of each constituent was accurately calculated. The use of this buffer (approximately 0.06 *M*) permitted the achievement of almost the same pattern of separation which has been reported by Moore and Stein *(1)* using sodium citrate buffer pH 3.42, 0.1 *M*, 0.9 × 100 (in column bed) operated at 37.5° C. However, this was accomplished in 9 to 10 hours (about 20% of the time required for the Moore and Stein technique for the same amino compounds). The adapted method follows:

Preparation of the ion exchange column. Pyrex glass chromatograph tubes (120 × 0.9 cm.) with ground joints (14/35) were used in this experiment. Dowex 50-X12 (200-400 mesh) as supplied in the hydrogen form was utilized. The conversion of the resin to the sodium form and the preparation of the column were made as described by Moore and Stein, except that for the preparation of the column the resin was washed on a Buchner funnel with a small amount of pH 4.0 sodium citrate buffer. A small piece of glass wool replaced the coarse sintered glass plate in the column, and the length of the resin bed was increased to 110 cm.

Preparation of the buffer. The pH of the buffer was determined by a Beckman glass electrode pH meter Model G. The buffer used in this experiment was a pH 4.0 sodium citrate buffer, composed of 0.056 moles of citric acid, 0.065 moles of sodium hydroxide, and 0.02 moles of sodium chloride per liter of solution. The buffer was made in large quantities and stored at 5° C., with thymol added as a preservative at the rate of 0.1%. Addition of 1 to 1.5% of a 50% aqueous solution of the detergent (BRIJ 35, Atlas Powder Co.) to the citrate buffer was satisfactory. This permitted faster flow rates without broadening the peaks on the effluent curves, which confirmed the observation of Moore and Stein.

Operation of 110 cm. column. A small aliquot portion (0.5-1.0 ml.) of the sample was adjusted to pH 3.0 and was delivered on the top of the column by using a slightly bent tip pipette to avoid disturbing the surface of the column. The column was then placed in position above an automatic constant volume (1.0 ml.) fraction collector. The fraction collector operates according to the principle of the collector, made by Varner and Bulen *(11)* and manufactured

by the Autonomous Instrument Company, Columbus, Ohio. The last of the buffer emptying from the volumetric siphon momentarily actuates a solenoid which permits a spring loaded table to advance one position.

Small aliquot portions (0.5 ml.) of sodium citrate buffer were used to wash the surface of the resin. The column was then connected to the buffer reservoir, which was adjusted to a level about 80-70 cm. above the top of the resin. This gave a flow rate of 1.0 ml. per 3-4 minutes. All results presented in this report are based on this flow rate.

The effluent was collected in 1.0-ml. fractions in small glass vials having a capacity of 10 ml., and the elution was stopped after collecting 150 fractions. All separations were conducted at room temperature, which varied from 21-32° C.

It was found necessary to establish for each new lot of resin the threshold volume for the elution of the various acidic compounds.

After completing the elution, the column was regenerated with 125 ml. of 0.2 *N* NaOH containing 1.0% of the BRIJ 35 detergent solution (Atlas Powder Company) followed by 125 ml. of the pH 4.0 sodium citrate buffer. In this regeneration step, sodium ions were displaced and the sulfonated polystyrene exchange resin was reconverted into the sodium form and ready for reuse.

Analysis of effluent fractions. About 5 μl. of each effluent fraction was spotted on Whatman No. 1 filter paper in the order of elution to determine the presence of the respective amino acids in the various tubes. The dry paper was sprayed, in a hood, with a ninhydrin-*n*-propanol solution, which was prepared as described by Moore and Stein *(5)*. After air drying, the paper was placed in an oven for 3 to 4 minutes at 100° C. to develop the color. By the location of ninhydrin positive fractions, the various amino compounds could be tentatively identified.

After establishing the presence of the amino acids in the effluent fractions, each fraction was adjusted to pH 5.0 with two drops of *N* NaOH for each 1-ml. fraction and analyzed by the photometric ninhydrin method of Moore and Stein *(5)*.

Three different lots of sulfonated polystyrene resin Dowex 50 were used in this study. Two of these were found to be satisfactory, but a third lot was unsatisfactory since no increase in the flow rate through a resin could be achieved and the separation of the amino compounds was incomplete. Microscopic examination of the first two lots revealed that they were similar in appearance, being homogeneous in respect to the size of the resin particles. The unsatisfactory lot of Dowex 50 had smaller polydispersed particles and a low porosity.

For each of the 11 amino compounds studied, a constant was determined from the standard curve based on the relationship between the optical density of the ninhydrin reaction product and the concentration of amino acids. These constants *(k)* are presented in Table 1. Except for proline, the *k* values agree with the data obtained by Moore and Stein. For cysteic acid, taurine, and glutamine, Beer's law was not obeyed for all concentrations. Curves resulting from the plot of extinction versus concentration for these amino acids were straight lines in the concentration range of 0.025 to 0.25 m*M*, with positive intercepts. For the remaining eight amino acids, Beer's law was obeyed up to concentrations

TABLE 1

Constants (k) obtained from the relation between optical density and concentrate of amino acids[a]

Amino acids	k	Amino acids	k
Cysteic[b]	4.28	Asparagine	3.60
Serine phosphate	4.20	Glutamine[b]	3.80
Taurine[b]	4.02	Glutamic	4.00
Aspartic	4.20	Proline	1.41
Threonine	4.00	Glycine	3.99
Serine	4.40	Leucine	4.13

[a] $C = \frac{D}{k}$

[b] The following relationship was found for 0.2 to 1.0 optical density:

$$C = \frac{D - 0.2}{k} + 0.025$$

Where: C = concentration of unknown in mM, D = optical density of the acid, and k = slope of the curve of extinction versus concentration.

of 0.25 mM. The reason for the deviation from Beer's law can not be explained at present, but some contaminant in the amino compounds may be responsible.

Mixtures containing 0.2 mg. of each of 11 acidic amino compounds in pH 4.0 buffer were adjusted to pH 3.0 and were separated by this modified ion exchange procedure. Replicate trials were conducted, and representative results are shown in Figure 1. Mixtures added to cheese and to starter cultures separated in the same manner as shown in Figure 1. The presence of the fermentation products did not shift the threshold volumes of the various compounds.

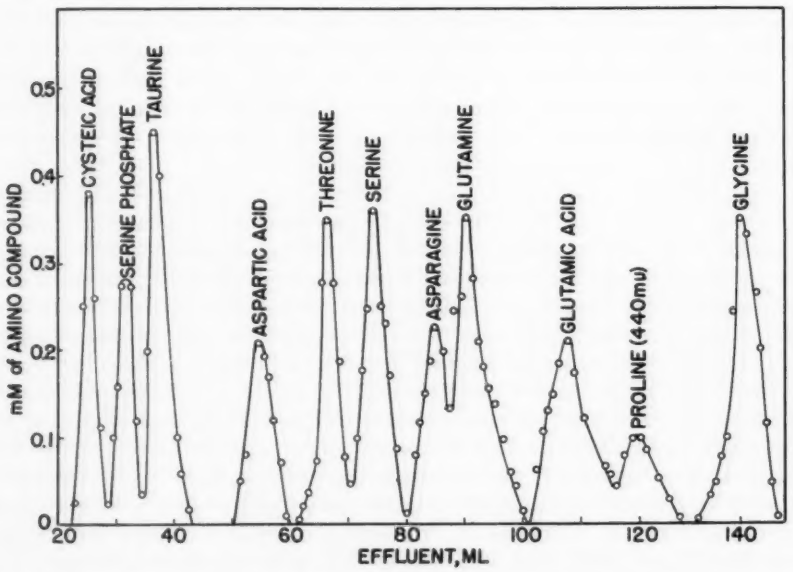


FIG. 1. Separation of eleven acidic amino compounds on a sulfonated polystyrene resin column: pH 4.0, flow rate of 1 ml. per 3 minutes.

In addition to the separation of these acidic compounds cited by Moore and Stein *(4)*, serine phosphate (phospho-serine), and glutamine also were identified.

Serine phosphate was identified after noting an unknown phosphate-containing compound in fermented milk cultures, *Lactobacillus bulgaricus* and *Lactobacillus lactis*, which was eluted just following cysteic acids. Serine phosphate obtained from the Eastern Regional Laboratory chromatographed in the same position, and mixtures of the unknown and known serine phosphate gave a symmetrical elution pattern. The unknown acid was collected from a number of starter cultures, hydrolyzed with hydrochloric acid, and rechromatographed. Only one elution peak appeared and this coincided with the known position of serine.

A second unknown compound in *Lactobacillus bulgaricus* starter cultures appeared which partially overlapped asparagine. A sample of L-glutamine (Nutritional Biochemicals Corporation) chromatographed in the same position, and a mixed chromatographic analysis of the unknown with glutamine gave a smooth symmetrical elution curve. Collection of the unknown was made from a number of analyses. A micro-Kjeldahl analysis was made, and the nitrogen content corresponded very closely to the calculated nitrogen content of glutamine.

TABLE 2

Recovery of eleven amino acids on a sulfonated polystyrene resin column: pH 4.0, flow rate 1 ml. per 3 minutes[a]

Amino compound	Concentration added	Recovery	
		Range	Average
	(mg.)	*(%)*	*(%)*
Cysteic acid	0.20	94-97	95.5
Serine phosphtae	0.20	100	100
Taurine	0.20	94-96	95
Aspartic acid	0.20	94-96	95
Threonine	0.20	88-92	90
Serine	0.20	99-101	100
Asparagine	0.20	85-90	87.5
Glutamine	0.20	95-97	96
Glutamic acid	0.20	89-93	91
Proline	0.34	93-97	95
Glycine	0.20	95	95

[a] Average of three trials.

The average recovery of the various pure amino compounds in a mixture is shown in Table 2. The data reveal that all of the compounds can be recovered quantitatively. The method was found to give reproducible results, with values on replicate analyses varying from the mean average by not more than $\pm 5.0\%$.

Application of the method was made to the analysis of free amino acids in cheese and bacterial cultures. Water soluble extracts on cheese cultures were obtained by the method of Sommer and Harper *(8)*. In order to concentrate the amino acids to a suitable concentration for analysis, the extracts were distilled under reduced pressure (20 mm.) at a reduced temperature (15-20° C.) to a volume of 5-10 ml. Analyses were made on the extracts and on the extracts containing an added mixture of amino acids (added to sample prior to concentra-

TABLE 3

The effect of cheese and starter cultures on the recovery of added amino acids (sulfonated polystyrene resin column: pH 4.0, flow rate 1 ml. per 3 minutes)[a]

Amino compound	Starter Culture[b]		Cheese[c]	
	% Recovery		% Recovery	
	Range	Average	Range	Average
Cysteic acid	89-94	91.5	86-96	92
Serine phosphate	96-101	97	97-99	98.5
Taurine	93-98	95	91-98	94.5
Aspartic acid	92-97	94	93-97	95
Threonine	90-93	91	88-94	90.5
Serine	97-102	99	92-100	97
Asparagine	87-92	90.5	85-96	91.5
Glutamine	91-96	95	93-101	97
Glutamic acid	92-98	94.5	89-97	93
Proline	91-96	93	92-99	94
Glycine	94-101	97	93-96	94.5

[a] Concentration of each compound was 0.1 mg.
[b] Average of 4 trials.
[c] Average of 6 trials.

tion). The threshold volume for any amino acid did not change, indicating that the fermentation products did not affect the qualitative elution of the amino acids. The results for recovery of added amino acids are shown in Table 3.

In general, the recoveries were similar to those found for pure acids in the absence of fermentation products. Some average recovery values were higher and some lower. The acid which showed the greatest reduction in recovery in the presence of fermentation products was cysteic acid. The type of fermentation product, cheese or culture, did not affect the reliability of the method as measured by recovery of added acids.

Although study of the neutral mono-amino mono-carboxylic acids was beyond the scope of this study, the effect of some of these acids on the separation of the acidic compounds was investigated. In Moore and Stein's original method, these acids were eluted just following the acidic compounds.

By using mixtures of acids, alanine, valine, and leucine were chromatographed with the 0.06 *M* pH 4.0 buffer at room temperature. The mixture was chromatographed alone and in the presence of the 11 acidic amino compounds. The results of replicate trials revealed that these acids did not interfere with the analysis of the acidic compounds. Alanine appeared between 142-154 ml. of eluent; valine was eluted in the 169-185 fractions; and leucine was eluted in 190-205 fractions. Only two trials were made to ascertain the recovery of pure acids, but the recovery of mixtures containing 0.2 mg. of each of the acids averaged 94, 95, and 92% for alanine, valine, and leucine, respectively.

DISCUSSION

The method presented in this study provides for more rapid quantitative analysis of only the acidic amino compounds. Nevertheless, it should serve a definite need in providing for more routine analysis of some of the more important amino compounds which are involved in cheese ripening, especially proline

and glutamic acid. Such methods are needed for studies of specific mechanisms which do not involve studies of the complete amino acid system of milk proteins.

The changes resulting in more rapid analyses of acidic amino compounds, which were made possible by slight modifications of Moore and Stein's original method, emphasize the statement of Moore and Stein that the use of Dowex 50 would be adaptable to many specialized techniques with relatively minor changes. In addition to the acidic acids, it seems possible that altering the condition of operation might be useful in analysis of the neutral and basic amino compounds. Limited evidence was obtained which indicated that the modified technique might also be applied to the separation of the neutral amino acids without changing the pH or concentration of the buffer and without increasing the temperature. Considerably more work is necessary to prove the reliability of this procedure and its applicability to analysis of cheese and cultured products.

Variations in the properties of different lots of Dowex 50 are a definite limitation to the method. In application of this method to Swiss cheese research a lot of resin was used which gave the same resolution patterns reported in this paper. However, the flow characteristics of the resin were such that it could not be operated at 1 ml/3 minute rate, but delivered 1 ml/6 minutes with the same head pressure used with the other lots of resin. The only difference in the resolution pattern was a complete separation of glutamine and asparagine. Slowing the flow rate of the resin lots used in this study to 1 ml/6 minutes resulted in the same separation pattern. The reason for the slower flow rate in the one lot of resin is not known at this time. The use of a slower flow rate for more complete separation of glutamine and asparagine would be useful for some studies, where isolation of these compounds is desired. However, for general application the increased flow rate of 1 ml/3 minutes is satisfactory and recommended.

The lower recovery of cysteic acid from cheese and cultures is probably due to interference of acidic ninhydrin positive substances as reported by Schram *et al. (7)*. However, the results indicate that cysteic acid could be measured quantitatively by this system. The failure of pure solutions of cysteic acid to follow Beer's law in low concentrations is not readily explained. The most plausible explanation would be that the cysteic acid standard contained some impurities.

The importance which Moore and Stein gave to the utilization of various criteria to the identification of amino compounds should be emphasized. During this study an observation was made that the complete absence of one acid could result in 2 to 5% shift in the threshold volume of the succeeding acids. Although this shift was never enough to cause a mistaken identity of the amino compound, the danger of misinterpretation of results should be realized. When this shift appeared, as mixture of the 11 amino compounds was chromatographed with the unknown sample, the resulting chromatogram showed that the presence of all the compounds restored the threshold volumes to the values obtained for the pure acids added as a mixture.

SUMMARY

The polystyrene ion-exchange chromatographic method of Moore and Stein *(4)* was modified to permit more rapid elution and to eliminate the need for temperature control. This was achieved by altering the pH of the buffer from 3.42 to 4.0 and the concentration of the buffer from 0.1 *M* to 0.06 *M*. These changes permitted more rapid elution, resulting in a shortening of the procedure by about 75%. In addition, the column could be operated between 20 and 30° C. without affecting the results.

Eleven acidic amino compounds were found to be separated by this method. In addition to those separated by Moore and Stein, serine phosphate and glutamine also were separated.

ACKNOWLEDGMENTS

The authors extend their sincerest appreciation to Quentin Van Winkle, Department of Chemistry, for assistance in arriving at the correct buffer concentration and to F. E. Deatherage, Department of Agricultural Biochemistry, for his suggestions leading to the identification of serine-phosphate; and to T. L. McMeekin of the Eastern Regional Research Laboratory, USDA, for supplying the synthetic serine-phosphoric acid.

REFERENCES

(1) Black, A. L., Kleiber, M., and Smith, A. H. Carbonate and Fatty Acids as Precursors of Amino Acids in Casein. *J. Biol. Chem.*, **197**: 365. 1952.

(2) Bryant, F. Chromatographic Studies of the Silk Fiber. II. Fractionation of Fibroin Hydrolyzate. *Textile Research J.*, **22**: 525. 1952.

(3) Hirs, C. H. W., Moore, S., and Stein, W. H. Isolation of Amino Acids by Chromatography on Ion Exchange Columns; Use of Volatile Buffers. *J. Biol. Chem.*, **195**: 669. 1952.

(4) Moore, S., and Stein, W. H. Chromatography of Amino Acids on Sulfonated Polystyrene Resins. *J. Biol. Chem.*, **192**: 663. 1951.

(5) Moore, S., and Stein, W. H. Photometric Ninhydrin Method for Use in Chromatography of Amino Acids. *J. Biol. Chem.*, **176**: 367. 1948.

(6) Partridge, S. M., and Brimley, R. C. Displacement Chromatography on Synthetic Ion Exchange Resins. VIII. A Systematic Method for the Separation of Amino Acids. *Biochem. J.*, **51**: 628. 1952.

(7) Schram, E., Moore, S., and Bigwood, E. S. Chromatographic Determination of Cystine as Cysteic Acid. *Biochem. J.*, **57**: 33. 1954.

(8) Sommer, H. H., and Harper, W. J. Methods for Studying the Ripening of Cheese. *J. Dairy Sci.*, **31**: 714 (Abs.). 1948.

(9) Stein, W. H., and Moore, S. Chromatographic Determination of Amino Acid Composition of Proteins. *Cold Spring Harbor Symposia Quant. Biol.*, **14**: 179. 1950.

(10) Tabor, H., and Hayaiski, O. The Enzymatic Conversion of Histidine to Glutamic Acid. *J. Biol. Chem.*, **194**: 171. 1952.

(11) Varner, J. E., and Bulen, W. A. An Automatic Constant Volume Fraction Collector. *J. Chem. Ed.*, **29**: 625. 1952.

(12) Wall, J. S. Simultaneous Separation of Purines, Pyrimidines, Amino Acids, and Other Nitrogenous Compounds. *Anal. Chem.*, **25**: 950. 1953.

A COMPARISON OF THE FERTILITY OF BULL SEMEN DILUTED IN EGG YOLK–CITRATE AND HOMOGENIZED MILK[1]

J. R. PERKINS, M. C. CARPENTER,[2] AND D. M. SEATH
Dairy Section, Kentucky Agricultural Experiment Station, Lexington

Boiled milk used as a semen diluter was first reported in 1950 by Michajilov *(5)*. In 1951, Thacker and Almquist *(9)* reported that on a limited number of services the fertility of semen diluted in boiled homogenized milk was equal to, or slightly higher than, that diluted with yolk–citrate. Since then other reports *(1, 2, 3, 4, 7, 8)* have indicated that milk which had received a high-heat treatment gave fertility results comparable to those with yolk–citrate.

Almquist and Thacker *(1)* found that six low-fertility bulls accounted for most of the improvement obtained with milk diluter over yolk–citrate. Dreher and Webb *(3)* also reported that bulls which tended to have the lowest per cent nonreturns on yolk–phosphate diluter gave the greatest response to milk as a diluter.

The present field study was conducted in order to *(a)* compare high-temperature treated milk with yolk–citrate as a semen diluter, *(b)* determine if there was an interaction between kind of diluter and age of the semen when used, and *(c)* determine if there was a between-bull nonreturn difference when comparing milk and yolk–citrate as diluters.

EXPERIMENTAL PROCEDURE

The experiment was conducted at the Kentucky Artificial Breeding Association during September, October, November, and December of 1953. Semen was collected weekly from bulls of the Jersey, Guernsey, Holstein, and Brown Swiss breeds. Semen was diluted 1:50, and alternate collections were diluted in homogenized milk heated to 97° C. for 10 minutes or in the conventional 1:4 yolk–citrate diluter. Streptomycin and penicillin were added to each of the diluters at the rate of 500 units each per milliliter of diluter.

Semen was shipped 4 days each week to 53 technicians. Approximately half of the cows were bred with semen 2 days old, and the remaining half were bred with semen 3 days old or older. Fertility results based on 60-90 day nonreturns were obtained from 19,939 first services during the experimental period. The analysis of variance *(6)* was used in an effort to determine sources of variation in fertility data.

RESULTS AND DISCUSSION

The average 60-90 day nonreturns for 19,939 first services during the 4-month experimental period (Table 1) was 70.3%. There were 9,927 services with egg

Received for publication August 2, 1954.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

[2] Manager, Kentucky Artificial Breeding Association.

TABLE 1

Per cent 60-90 day nonreturns for 2- and 3-day-old semen diluted with milk and with yolk–citrate

Diluter	2-day-old semen		3-day-old semen		Total	
	1st services	60-90 day nonreturns	1st services	60-90 day nonreturns	1st services	60-90 day nonreturns
		(%)		(%)		(%)
Yolk	4,799	73.7	5,128	68.9	9,927	71.2
Milk	4,797	75.3	5,215	64.1	10,012	69.4
Total	9,596	74.5	10,343	66.6	19,939	70.3

yolk diluter resulting in a 71.2% nonreturn rate and 10,012 services with milk diluter having a 60-90 day nonreturn rate of 69.4%. The difference of 1.8% in favor of yolk was not statistically significant.

Milk as a diluter was superior to the yolk–citrate diluter when used with 2-day-old semen (Table 1), the 60-90 day nonreturns being 75.3% and 73.7%, respectively. For the 3-day-old semen the 60-90 day nonreturns with yolk diluter dropped 4.8%, whereas with milk diluter the decrease was 11.2%. This difference between per cent nonreturns for 2-day-old and 3-day-old semen was found to be highly significant, as was the interaction of age of semen and diluters, i.e., the milk diluter was best for 2-day-old semen and the yolk–citrate was superior for 3-day-old semen.

The majority of the services, 19,308, or 97%, were with semen collected from 31 bulls. In considering differences between bulls, four of the original 35 bulls were omitted from the study because of limited numbers of services. Of the 31 bulls compared (Table 2), 14 showed an increase in per cent 60-90 day nonreturns with milk diluter as compared to their nonreturns on yolk–citrate during the same period. These bulls had a nonreturn rate of 67.9% with 3,506 services on yolk diluter and 71.1% with 3,693 services on milk diluter. Two of the bulls showed no change. The other 15 bulls had a lower nonreturn rate on milk than on yolk, i.e., 5,482 services with 68.2% and 5,455 services with 73.4%, respectively. The difference was found to be statistically significant. There was a tendency for the semen with the lower fertility on yolk–citrate diluter to be improved with milk diluter, whereas that with the higher fertility on yolk diluter tended to be lowered somewhat with a milk diluter.

The convenience of preparing the milk, plus the financial savings, as compared to yolk–citrate, tended to favor the use of the homogenized milk diluter. Even with these advantages in favor of milk, there appeared to be instances when it should not be used. The results of this experiment indicate that before changing to milk diluter exclusively, one should consider the age of the semen to be used and should run a comparison on all bulls in order to determine whether or not each bull's semen will give satisfactory results with milk as a diluter. It is probable that for the bulls whose semen shows a decrease in fertility when diluted with milk, yolk–citrate should be continued.

TABLE 2

Fertility of bulls on yolk–citrate and milk diluters and the change in per cent 60-90 day nonreturns caused by milk diluter

Bulls	Yolk		Milk		Change with milk	
	No. 1st services	N-R	No. 1st services	N-R		
		(%)		(%)	(%)	
323	162	60.5	188	70.8	+10.3	IMPROVED WITH MILK DILUTER
464	157	63.7	206	71.8	+ 8.1	
542	370	61.1	390	66.2	+ 5.1	
202	565	71.7	663	75.9	+ 4.2	
645	145	71.7	91	75.8	+ 4.1	
305	164	57.9	207	61.4	+ 3.5	
661	103	68.9	83	72.3	+ 3.4	
641	334	56.9	351	59.8	+ 2.9	
422	328	74.4	296	77.1	+ 2.7	
662	90	78.9	74	81.1	+ 2.2	
49	314	72.9	343	74.4	+ 1.5	
243	299	68.9	312	70.2	+ 1.3	
462	191	76.4	182	77.5	+ 1.1	
501	284	69.0	307	70.1	+ 1.1	
421	412	73.1	378	73.1	—	NO CHANGE
423	203	73.9	179	73.8	—	
644	133	72.9	107	46.7	−26.2	LOWERED WITH MILK DILUTER
461	90	87.8	92	69.6	−18.2	
643	323	67.2	259	55.2	−12.0	
504	582	76.6	635	67.1	− 9.5	
324	326	67.5	361	58.2	− 9.3	
561	118	72.9	109	65.1	− 7.8	
301	145	71.1	213	63.4	− 7.7	
663	92	77.2	91	72.5	− 4.7	
221	350	70.3	305	66.6	− 3.7	
523	433	69.7	416	66.1	− 3.6	
524	416	73.3	511	69.9	− 3.4	
543	241	75.5	182	73.1	− 2.4	
503	860	78.3	895	76.1	− 2.2	
304	964	74.7	996	72.6	− 2.1	
204	382	67.1	310	65.8	− 1.3	

SUMMARY

When homogenized milk was heated at 97° C. for 10 minutes and then used for bull semen, no significant differences were found in 60-90 day nonreturns between it and the standard yolk–citrate diluter. A highly significant difference was found in the per cent nonreturns between 2-day-old and 3-day-old semen with milk and yolk diluters. Milk diluter gave the best results with 2-day-old semen and yolk–citrate diluter was best with 3-day-old semen. There was a drop of 4.8% from 2-day-old to 3-day-old semen with yolk–citrate and 11.2% drop with milk.

There was also a statistically significant difference between bulls in per cent nonreturns. The tendency was for the bulls of lower fertility to show improvement in per cent nonreturns when milk diluter was used.

REFERENCES

(1) ALMQUIST, J. O., AND THACKER, D. L. Fertility of Bull Semen Diluted with Boiled, Homogenized Milk and Boiled Skim Milk. *J. Animal Sci.*, 11 (4): 787. (Abs.) 1952.

(2) COLLINS, W. J. Evaporated Milk as a Semen Extender. *J. Dairy Sci.*, 36: 578. (Abs.) 1953.

(3) DREHER, W. H., AND WEBB, J. H. A Comparison of Homogenized Whole Milk and Egg Yolk–Phosphate as Diluters for Bull Semen. *J. Dairy Sci.*, 36: 673. 1953.

(4) FLERCHINGER, F. H., ERB, R. E., AND EHLERS, M. H. The Use of Heated Homogenized Milk as a Diluter for Bull Semen. *J. Dairy Sci.*, 36: 1016. 1953.

(5) MICHAJILOV, N. N. Sperm Dilution in the Milk. *J. Am. Vet. Med. Assoc.*, 117: 337. (Abs.) 1950.

(6) SNEDECOR, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames. 1946.

(7) SOBEK, V. Milk as a Diluent for Bull Semen for Artificial Insemination. *Animal Breeding Abstr.*, 22 (1): 46. (Abs.) 1954.

(8) THACKER, D. L., AND ALMQUIST, J. O. Milk and Milk Products as Diluters for Bovine Semen. *J. Animal Sci.*, 10: 1082. (Abs.) 1951.

(9) THACKER, D. L., AND ALMQUIST, J. O. Diluters for Bovine Semen. I. Fertility and Motility of Bovine Spermatozoa in Boiled Milk. *J. Dairy Sci.*, 36: 173. 1953.

ALUMINUM IN COW'S MILK[1]

J. G. ARCHIBALD
Massachusetts Agricultural Experiment Station, Amherst

This paper is the seventh in a series on the mineral elements of cow's milk *(1, 2, 3, 4, 5, 6)*. The possible toxicity of aluminum dissolved by the action of milk and other foods from cooking utensils and from commercial processing and packaging equipment has been extensively investigated *(7, 11, 14, 15, 16, 17, 18, 20, 23, 26)*. The general conclusion has been that the amounts so dissolved are so small as to be of no significance toxicologically or with respect to destruction of vitamin C in the pasteurization of milk *(12, 19, 21)*. Very little work has been done on the amount of aluminum (if any) naturally present in milk or on the effect of feeding supplements of the element on its level in milk.

Previous investigators with one exception *(24)* have reported the presence of aluminum in milk but have not determined the amount *(8, 9, 10, 25, 27)*. Underhill *et al. (24)* report that a sample of fresh milk contained 1.54 mg. of the element per 100 g. of milk. This investigation was undertaken to obtain information on these points. If the data showed any considerable amounts of aluminum in milk as a result of feeding a supplement, the possible significance of the problem in terms of human and animal nutrition would later be investigated.

EXPERIMENTAL

An earlier paper *(1)* has described the procedure in connection with the feeding trials. These were conducted during the winter of 1953-54, with six cows allotted to two sub-groups of three each, representing the Ayrshire, Holstein, and Jersey breeds. Each breed pair was matched as closely as possible with respect to stage of lactation. The supplement fed was aluminum potassium sulfate [$Al\,K\,(SO_4)_2 \cdot 12\,H_2O$] in an approximate daily amount of 2 g., equivalent to 114 mg. of elemental aluminum. One group received the supplement during December and January, the other during February and March. No ill effects of feeding this amount of alum were evident.

Composite 2-day milk samples of 1 l. each were taken from each cow once a month at about the mid-point of the month. Triplicate 300-ml. aliquots were evaporated to dryness and ashed in an electric furnace at dull redness (approximately 550° C.); the ash was then dissolved in 50 ml. of HCl (1:3). Aluminum was determined photometrically[2] by a method described by Hillebrand *et al. (13)*. The authors of the method do not indicate its reproducibility; accordingly, a test to ascertain this was the first step in the work reported here. Triplicate 300-ml. aliquots of mixed herd milk from cows not receiving an aluminum sup-

Received for publication August 7, 1954.

[1] Contribution No. 962 of the Massachusetts Agricultural Experiment Sation.

[2] The instrument used was a Model 11 Coleman spectrophotometer. Measurements were made at a wave length of 525 mμ with Corning filter ‡PC5.

plement were carried through the recommended procedure and were found to contain 0.34 mg. of aluminum per liter of milk. Known amounts of a stock solution of potassium alum were then added to further 300-ml. aliquots of the same lot of milk in such quantity that for two of the aliquots the total amount of aluminum present was doubled (0.68 mg. per liter) and for two more the amount was tripled (1.02 mg. per liter). The results in Table 1 indicate satisfactory recovery.

TABLE 1

Reproducibility of method for aluminum

In herd milk	Added to herd milk as potassium alum	Total in sample	Total recovered	Recovery of added Al	Recovery
(Parts per million of aluminum)					*(%)*
0.34	0.34	0.68	0.700	0.360	105.9
0.34	0.34	0.68	0.675	0.335	98.5
0.34	0.68	1.02	1.034	0.694	102.0
0.34	0.68	1.02	1.034	0.694	102.0
				Average recovery—102.1	

TABLE 2

Effect on aluminum content of milk of feeding cows aluminum potassium sulfate

	Cows on control ration				Cows receiving supplemental aluminum			
	(milligrams of aluminum per liter of milk)							
	1st half of season							
	A70[a]	H72	J54	Average	A69	H46	J50	Average
December	0.35	0.65	0.35	0.45	0.49	1.36	0.79	0.88
January	0.15	0.28	0.45	0.29	0.85	0.39	0.57	0.60
Av., 1st half	0.25	0.47	0.40	0.37	0.67	0.88	0.68	0.74
	2nd half of season							
	A69	H46	J50	Average	A70	H72	J54	Average
February	0.16	0.31	0.45	0.31	0.78	0.53	0.66	0.66
March	0.52	0.89	0.97	0.79	0.87	1.00	1.42	1.10
Av., 2nd half	0.34	0.60	0.71	0.55	0.83	0.77	1.04	0.88
Av., entire season	0.30	0.54	0.56	0.46 ±.08[b]	0.75	0.83	0.86	0.81 ±.09[b]

[a] The initial letter prefixed to each cow's number indicates the breed.
[b] Standard error of the mean. See Snedecor, pages 54 and 58, *(22)*.

RESULTS

The values are summarized in Table 2. The amounts of aluminum in the milks varied considerably from month to month and between individuals. The average per liter of milk was 0.46 mg. (range: 0.15–0.97) when the cows were fed the control ration and 0.81 mg. per liter (range: 0.39–1.42) when they received the supplement. With two clear-cut exceptions[3] (H46 and J50—Janu-

[3] There is a third exception (A69—December *cf* March), but strictly speaking it is not as valid as the others because it compares a value for 1st month on supplement with one for 2nd month on control. This increase occurred in March when, as noted elsewhere, there was a marked increase in the aluminum content of all milks irrespective of group.

ary *cf* March) the milks contained more aluminum when the supplement was fed, the average increase being 0.35 mg. This difference was significant *(22)* at the 5% level ($t = 2.917$, 11 df).

These values are very much lower than the single one reported by Underhill *(24)*. Converted to the same basis as ours (milligrams per liter), his figure would be approximately 15 mg. Possibly the difference represents further refinement of techniques for determining trace amounts of aluminum since his paper was published in 1929. The marked increases in aluminum content of the milk in March in both groups are not easy to explain. Possibly they are due to increased amounts of aluminum in the regular ration; if so, most probably these were in the roughage. Analytical error is rendered improbable by the facts that *(a)* all determinations were made by one individual and *(b)* the February and March samples were all analyzed in the space of three consecutive days.

It appears that aluminum is a natural constituent of cow's milk, occurring in amounts of the order of 1 mg. per liter or less. Feeding an aluminum supplement resulted in rather consistent, although not large, increases in the amount present.

SUMMARY

Milk from six cows fed alternately for a 4-month period on a control ration and one containing an aluminum supplement (potassium alum), was analyzed photometrically for its aluminum content. The control samples contained on the average 0.46 mg. of aluminum per liter; the samples obtained when the cows received the supplement averaged 0.81 mg. per liter. The average difference of 0.35 mg. was significant at the 5% level.

REFERENCES

(1) Archibald, J. G. Manganese in Cows' Milk. *Milk Plant Monthly*, 30: 36. 1941.
(2) Archibald, J. G. Zinc in Cows' Milk. *J. Dairy Sci.*, 27: 257. 1944.
(3) Archibald, J. G. Cobalt in Cows' Milk. *J. Dairy Sci.*, 30: 293. 1947.
(4) Archibald, J. G. Nickel in Cows' Milk. *J. Dairy Sci.*, 32: 877. 1949.
(5) Archibald, J. G. Molybdenum in Cows' Milk. *J. Dairy Sci.*, 34: 1026. 1951.
(6) Archibald, J. G., and Lindquist, H. G. Manganese in Cows' Milk. *J. Dairy Sci.*, 26: 325. 1943.
(7) Bertrand, G., and Serbescu, P. The Toxicity of Aluminum as Compared to that of Iron, Nickel, and Other Metals (Translated title). *Compt. rend.*, 193: 128. 1931.
(8) Bleyer, B., and Kallmann, O. Some Little Known Constituents of Milk. *Biochem. Z.*, 155: 54. 1925.
(9) Dingle, H., and Sheldon, J. H. A Spectrographic Examination of the Mineral Content of Human and Other Milk. *Biochem. J.*, 32: 1078. 1938.
(10) Drea, W. F. Spectrum Analysis of Milk Ashes. *J. Nutrition*, 8: 229. 1934; and 16: 325. 1938.
(11) Flinn, F. B., and Inouye, J. M. Metals in Our Foods. *J. Am. Med. Assoc.*, 90: 1010. 1928.
(12) Gwyer, A. G. C. Separation of Aluminum as Phosphate in the Presence of Calcium Phosphate with Special Reference to the Action of Milk on Aluminum. *Analyst*, 57: 704. 1932.
(13) Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., and Hoffmann, J. I. *Applied Inorganic Analysis*. 2nd ed. John Wiley and Sons, New York. 1953.

(14) Intonti, R. Use of Aluminum Kitchen Utensils. (Translated title). *Giorn. chim. ind. appl.*, **16**: 159. 1934.

(15) Lehmann, K. B. Critical and Experimental Investigation of Aluminum Utensils from the Standpoint of Hygiene. *Arch. Hyg.*, **102**: 349. 1929.

(16) Massatsch, C. The Behavior of Some Various Grades of Aluminum Under the Action of Water from Different Sources. (Translated title). *Hauszeitshr. V.A.W. Erftwerk A. G. Aluminum.*, 1: 75. 1929.

(17) Mitchell, C. A. D. Aluminum Vessels and Food Contamination, I, II. *J. Roy. Army Med. Corp.*, **61**: 99 & 193. 1933.

(18) Monier-Williams, G. W. Aluminum in Food. *Repts. Pub. Health and Med. Subjects*, No. 78. 1935.

(19) Schieblich, M. The Effect of Metals on the Vitamin Content of Milk. (Translated title). *Deut. Nahrungsm. Rundschau.*, **19**: 150. 1932.

(20) Scholtz, H. Present Knowledge of the Use and Storage of Milk and Milk Products. (Translated title). *Gesundh. Ing.*, **53**: 44. 1930.

(21) Schwartze, E. W., Murphy, F. J., and Cox, G. J. Effect of Pasteurization upon the Vitamin C Content of Milk in the Presence of Certain Metals. *J. Nutrition*, **4**: 211. 1931.

(22) Snedecor, G. W. *Statistical Methods.* 2nd ed. Collegiate Press, Ames, Iowa. 1938.

(23) Thieme, A. Suitability of Pure Aluminum Vessels for Culinary Purposes (Translated title). *Chem. Ztg.*, **53**: 973. 1929.

(24) Underhill, F. P., Peterman, F. I., Gross, E. G., and Krauss, A. C. The Metabolism of Aluminum. VII. The Aluminum Content of Some Fresh Foods. *Am. J. Physiol.*, **90**: 72. 1929.

(25) Wright, N. C., and Papish, J. The Inorganic Constituents of Milk. *Science*, **69**: 78. 1929.

(26) Wührer, J. The Hygienic Significance of Aluminum Cooking Vessels. (Translated title). *Arch. Hyg. u. Bakteriol.*, **112**: 198. 1934.

(27) Zbinden, C. The Infinitely Small Quantities of Certain Elements in Milk and Their Detection by the Spectrographic Method. *Lait*, 11: 114. 1931.

PROVING DAIRY SIRES AND DAMS[1]

JAY L. LUSH AND LON D. McGILLIARD
Department of Animal Husbandry, Iowa State College, Ames

To "prove" a dairy sire is to estimate his breeding value, which is never observable directly, from the observed qualities of his known offspring. Since these qualities are affected by many other things, the offspring indicate the breeding values of their parents only imperfectly. The various errors which can thus creep into the "proof" of a sire or dam can generally be reduced (in varying degrees) by such procedures as correcting for known peculiarities of the environment, increasing the number of offspring, and comparing the offspring with the observed qualities of the other parents or of still other relatives or of their contemporary herd mates. The problem of "proving" is to use each such procedure in a way that the breeding values will be estimated as accurately as is possible with the available information. The papers on proving dairy sires are far too numerous to list completely. Only articles which still seem to be pertinent and a few which, although now obsolete, have been cited widely are listed here.

PRINCIPLES INVOLVED

Although progeny tests in actual practice are always based on several offspring, the way in which various procedures can diminish the errors in the proof can be seen most clearly by considering first how the record of one daughter indicates her sire's breeding value. A simple way to express this is:

$$y = s + b + r \qquad (1)$$

where the letters have the following meanings:

- y is the difference between this daughter's record and the breed average;
- s (for sire's transmitting ability) is the amount by which this sire makes the average of his daughters higher or lower than the breed average;
- b (for bias) is the amount by which the daughters of a sire are above or below the breed average because of any circumstances, other than this sire's breeding value, which are alike for all his daughters; and
- r (for random) is the amount by which this one daughter's record was higher or lower than the records of her paternal sisters.

Since y, s, b, and r all indicate deviations from averages, each of these four variables will sum to zero when several sires each with many daughters are considered, and its plus values and minus values will be about equally frequent.

We wish to know s, but only the record (y) can be observed directly. The many things which can make the daughter's record not indicate her sire's breed-

Received for publication August 13, 1954.

[1] Journal Paper No. J-2576 of the Iowa Agricultural Experiment Station, Ames. Project No. 1053. This review was prepared at the suggestion of the Purebred Dairy Cattle Association.

ing value accurately are divided into b and r because averaging the records of unselected daughters of a sire will reduce the errors which are grouped in r but will not reduce at all the errors which constitute b. Other procedures, such as comparing the daughter's record with that of her dam, will usually (although not necessarily) reduce the errors from b but increase those from r.

The largest part of b comes from environmental conditions which are identical for all daughters of the same sire but vary from one sire to another. Differences among sires in the average breeding value of their mates can also contribute something to b. This b is a constant for all daughters of the same sire but is a variable from sire to sire.

Most of r comes from environmental effects which vary from lactation to lactation for the same cow and differ among contemporary herd mates. This is shown by the intraherd repeatability of lactation fat or milk records generally being around 0.4 to 0.5. But r also contains any environmental effects which are permanent for a daughter through her lifetime but vary among the daughters of the same sire. An injury causing permanent damage to the udder prior to first calving is an example. Also, r contains the differences caused by the sampling nature of inheritance, whereby some daughters receive one gene and some receive the other from each pair of genes heterozygous in a parent.

Differences caused by dominance, or by epistasis, or by interactions between heredity and environment are largely in r but can be partly in b. Dominance and epistatic differences result from genes having effects when in some combinations which they do not have when in other combinations. Interaction between heredity and environment occurs when the same genetic difference causes a larger phenotypic difference under one environment than under another environment.

Differences in breeding values of the sire's mates and differences caused by the dominance deviations would be wholly in r if the mates of each sire were a random sample of the breed. Actually the mates of a sire usually will be related to each other a little, several being half sisters and a few related in other ways such as daughter and dam, or full sisters, or aunt and niece. Too, the mates of one bull may have been selected more intensively or toward a different goal than the mates of other bulls. This can put much of the differences between dams and a little of the effects of dominance into b.

A small part of the epistasis is included in b, even if each sire is mated to a random sample of cows, since daughters of a sire tend to receive from him a fraction of the special combinations of non-allelic genes. If his mates are related to each other or if they are selected toward a goal uncommon in that breed, more of the epistasis will go into b, although most of the epistasis will still be in r except under extremely unlikely circumstances, such as each bull being mated to a different highly inbred line of cows.

The effects of interactions between heredity and environment will be partly in b because the daughters of a sire will not include the full range of genotypes possible in a whole breed and they usually will not be exposed to the full range

of environments prevailing in the whole breed. Therefore, the interaction effects on paternal sisters will be correlated at least a little.

BIASES FROM SELECTING DAUGHTERS OR MATES OR RECORDS

Daughters. To use only the "best" daughters selects those daughters for whom the r elements happened to be more favorable than average. That large biases are introduced by selecting the daughters is shown by the following figures[2] for a bull whose 15 daughters have one record each, with the standard deviation among the records of paternal sisters being 80 lb. of fat. The average of the n daughters with the largest records will be above the average of all 15 by about the following amounts:

n	*Bias (in lb.)*
14	10
12	26
10	41
8	57
5	83

Approximate allowance for the average bias caused by omitting daughters with low records could be made by using these figures, but the actual bias would vary from sire to sire.

The ideal that the daughters shall be absolutely unselected cannot be achieved completely. Natural selection, such as the death of daughters bearing lethal genes or the failure of hereditarily barren daughters to freshen *(68)*, can have affected the data, but probably such things are moderately rare. The herdsman may have culled a few runty or unthrifty heifers before they had a chance to freshen. A heifer may have freshened but produced so poorly that she was discarded within 2 or 3 months and the facts about her may never appear in the proof. Whether selection of daughters was intense can be guessed if the number of daughters in the proof can be compared with the number known to have been born and to have lived long enough that they should have records. Such information is usually difficult to get. More complete reporting and use of records of incomplete lactations would reduce substantially this bias from selecting daughters.

Mates. Selection of the sire's mates (the dams of the daughters in the proof) will generally have been more intense than selection of the daughters, but it introduces much less bias *(44)*. The difference is that the records on which the daughters were selected are in the daughter average, whereas only half of the genic superiority achieved in the mates by their selection appears in the daughter average. When mates' records are subtracted from the daughters' records, as

[2] Based on Table 20 of the statistical tables by Fisher and Yates. The distribution of butterfat records is near enough to "normal" that this table gives a close approximation to the actual biases.

in a sire index, selection among the mates will have more complex effects since the average records of those cows which survive the selection and appear as dams will be higher than their transmitting abilities. However, subtracting the mates' records will go far toward correcting the usually large errors in b from herd-to-herd differences in environment, if mates and daughters were kept in the same herd and managed alike.

Records. To use only the highest record of the cow is to seek out that record made in the best environment yet experienced by that cow but without knowing what that environment was *(10, 13, 24)*. To use this highest record to predict what the cow or her relatives will do in the future is to predict implicitly that they will continue to get an environment better than average by the same unknown amount. That this bias is large can be seen from the following figures which show the average bias when the standard deviation among records of the same cow is about 60 lb. of fat.

Intensity of selection	*Amount of bias in pounds*
Highest record out of two	34
Highest record out of three	51
Highest record out of four	62
Highest record out of five	70
Highest two out of three	26
Highest two out of four	40
Highest two out of five	50
Highest three out of four	21
Highest three out of five	33
Highest four out of five	17

An individual buyer might use such figures as these to discount selected records which are quoted to him, but in such small samples as two to five records, this discount could be much too small in some individual cases and much too large in others.

Using the best record is especially unfair for comparing individual cows which do not have the same number of records *(8)*. Suppose three cows actually have the same real ability, but one of them has only one record, one has two records, and one has five records, and the intra-cow standard deviation is 60 lb. Then, on the average, using only the best record of each will make the cow with five records appear 70 lb. better as a producer than the cow with only one record, and 36 lb. better than the cow with two records.

Unfairness from using selected records is generally less extreme for comparing sire proofs than for comparing cows since a daughter average involves several cows and the average number of records per cow for such groups will not vary widely from sire to sire. Selecting the best record will generally bias the average of the sire's mates more than it will bias the average of his daughters because the daughters will be younger when the proof is assembled and will not yet have had as many chances to make high records.

"Abnormal" records. In principle, a record should be discarded when it was made under circumstances likely to have affected the record so strongly and unpredictably that this record is almost useless for predicting the cow's transmitting ability or her producing ability in another lactation. Discarding should be based only on clearly recognizable and specific circumstances other than the size of the record. To use the size of the record itself to indicate when circumstances were "abnormal" enough that the record should be discarded would open the door to most of the biases which come from selecting only the high records for use. An owner or herdsman might, for his own guidance, make some allowance for a cow having had some illness, such as milk fever or mastitis, but it is impossible to be sure how much allowance to make in any such case. It is easy to deceive oneself unknowingly by looking for excuses to discard whenever the record is low but by failing to look for the same circumstances if the record is high. In any event, it is not practical for officials of breed associations or public agencies to make such allowances when proving sires or dams.

WAYS OF MAKING SIRE PROOF MORE ACCURATE

1. *Standardizing environment* can reduce the error. Genes or breeding values are known only by their phenotypic expressions, and these can be modified by the kind of environment which prevails. The very same genotype which would cause a cow to produce 400 lb. in one environment might make her produce 500 lb. in some other environment and only 350 lb. in yet a third environment. Any numerical measurements or definitions of producing or transmitting ability must (whether explicitly stated or not) assume some level or set of environmental conditions as the standard ones under which that numerical value will occur.

If it were physically and financially possible to control the environmental conditions so closely that for every cow in every lactation the conditions actually were standard; or if those conditions were not controlled physically but could be corrected perfectly for the effects of all differences between the actual conditions and the standard ones, then all of the records of a cow would be identical. One lactation record would tell as much about her as any other or as the average of all her lactations. When such a record was used as a proof of her sire, the only sources of error would be Mendelian segregation, the breeding value of her dam, and the vagaries of dominance and epistasis.

Perfect control of the environment or perfect correction for the effects of such environmental variations as did occur is impossible, but since environmental sources are usually responsible for much more than half of the variance in single records of milk and fat *(36)*, any inexpensive physical control or statistical correction which actually does reduce this variation considerably is likely to be worth while. It might be imagined that diligent efforts to standardize the actual environments could make variations in them unimportantly small, but no one has ever demonstrated, by achieving high repeatability of individual records over several years, that this actually can be done, even if the expense of such physical control could be ignored. Standardizing the length of lactation and

correcting for differences in age and in frequency of milking are generally agreed to be worth while, but there is little agreement on the wisdom of making other corrections *(15)*. Likewise, there is little evidence on how much actually could be achieved by attempting to standardize physically the environment of whole herds more than is generally done in D.H.I.A. or H.I.R. herds.

Individuals studying sire proofs often find it profitable to visit the herds and study the barn books or otherwise reach some opinion about the average kinds of environment under which the records were made. Grades or scores for feeding practices or for other environmental factors have been correlated moderately with production in some cases *(4, 51)*, but these studies have not yet resulted in procedures by which a breed association or D.H.I.A. official could correct sire proof more nearly to what it would have been under standard environmental conditions.

The question of what kind of environment should be used as the standard one is still debated occasionally. Common sense indicates that cows should be kept and tested under the kind of environment under which they and their descendants are to be used commercially, but the practical importance of following this guiding principle depends on whether interactions between heredity and environment are large and frequent. The facts yet reported, such as the general sameness of repeatability and heritability at different levels of production *(36)*, agree best with the idea that environmental regimes which cause higher production generally multiply both the environmentally caused and the genetically caused differences between herd mates at somewhere near the same rates. This makes the differences between herd mates larger in absolute units when the production level is high but relatively little more or less reliable for purposes of selection between them. The importance of interactions between heredity and environment is one of the least explored questions in dairy breeding.

2. *Increasing the number of daughters* from one to four reduces sharply the error from r, which is the major source of error when n is small *(8, 36, 51)*. This, of course, raises the accuracy of the daughter average as an indicator of the sire's breeding value. But increasing n does nothing to decrease the errors from b which, if they are at all large, become the main source of error remaining after n is much larger than 3 or 4. Therefore, increasing n above 4 may make only small increases in accuracy. The formula which shows this increase in accuracy with increasing n is that the correlation between y_n and s is:

$$\frac{S}{S + B + R/n} \qquad (2)$$

where the capital letters indicate the respective individual variance components caused by s, b, and r. Contrast the situation in which the ratio, $S{:}B{:}R$, is 1:5:14 (which might well occur in actual data where the sires were proved in different herds and correction for environmental differences between herds was made only by subtracting the average of the sire's mates as is done in the "equal-parent" or "regression" indexes) with that in which the bias is first removed to make this ratio 1:0:14. The correlation between the sire's breeding

value and the average production of his n daughters then goes up with n as follows:

n	*Bias present*	*Bias absent*
1	0.22	0.26
2	0.28	0.35
3	0.31	0.42
4	0.32	0.47
10	0.37	0.65
20	0.39	0.77
00	0.41	1.00

The presence of this bias makes little difference in the accuracy when there is only one daughter but does prevent the accuracy from rising rapidly with n, especially after n passes 3 or 4. If the bias were first removed, raising n from 1 to 4 would nearly double the accuracy but would still leave it far below the limit it could reach. If no bias from b exists and all of the errors are in r, considerable accuracy can be gained by increasing the number of daughters even past 10 or 20. In practice, where at least a few errors from b remain, little accuracy is gained by increasing n past 10 *(39)*.

Many studies of the number of daughters required to prove a sire have really asked of the data only the correlation between the average of the records of the first n daughters and the average of m other daughters *(17, 23)*. In effect, this is asking the repeatability of the daughter average rather than its real accuracy as an indicator of the transmitting ability of the sire. This repeatability, the correlation between y_n and y_m, is simply:

$$\frac{S + B}{S + B + R/n} \qquad (3)$$

when n and m are equal. It includes the biased errors in the accuracy. If they are important, this procedure makes the daughter average seem far more accurate for sire proof than it really is. Occasionally this has been distorted even more by asking how nearly the average of the first n daughters is like the average of the first t daughters when the t includes the n *(14)*, or asking how much the proof changes when n increases to t. This contains so much automaticity that the correlation would be $\sqrt{\frac{n}{t}}$, even if the first daughters of a bull indicated nothing at all about what his future daughters would do.

When bulls are proved in artificial breeding associations *(57, 58, 59)*, b is also reduced with increases in n, since the major portion of b comes from things which differ from one herd to another. Yet, such parts of b as are caused by conditions which prevail over the whole region in which a sire is used artificially, but not over other regions from which come the data on other sires, would not be reduced. For example, New York, Iowa, and Oregon may differ noticeably in average feeding, management, or climate. If each artificially proved sire had been used within only one of these regions, then the effects of the environmental

differences between regions would not be diminished by making n large but would remain in b to mislead one who supposed that the differences between the daughter averages of these bulls were due wholly to qualities of the bulls themselves.

Setting a definite number of daughters as the minimum for proving a sire is arbitrary but must be done if an official scheme is to operate simply and be widely understood. Accuracy is not the only consideration. If the proving is to be much help in breed improvement, many more bulls should be proved than will actually be needed for use after the proof is known. Otherwise one cannot choose for further use only the bulls with the very highest proofs. The proof should be available for action while the bull is still alive and healthy. An important argument for setting the official number as low as five is that thus one can prove more bulls and prove them younger than if more daughters were required. The lower accuracy in the proof on five (which will be only a little lower if b was important) may be more than offset by the opportunity to select more intensively and earlier. Requiring too many daughters could lower the net rate of progress. For example, if 10 bulls are needed and 500 daughters could be used to prove the promising ones, one could prove 20 bulls with 25 daughters each, 50 bulls with 10 daughters each, or 100 bulls with 5 daughters each. The first would be more accurate proof for each bull, but when the results were in, the apparently best half of all bulls tested would have to be used; whereas in the middle case only the apparently best 1/5, and in the last case only the apparently best 1/10, would be needed. Under most conditions the extra intensity of selection possible in the middle case would more than offset its lower accuracy as compared with the first case, but the still higher intensity in the third case might not be enough to offset its lower accuracy.

3. *Records of the sires' mates* can be used to correct for differences in the average breeding values of the mates of various bulls and also for such environmental effects as are alike for the mates and daughters of a sire but differ from one sire to another. If the mates of each sire were truly a random sample of the breed and if b were zero, proof by n daughter-dam pairs would be as accurate as proof by $n + 1$ daughters from untested dams *(41)*. Actually the mates are rarely a wholly random sample and b often is large; hence, use of the mates' records is generally more helpful than this.

Use of the mates' records does not invariably lead to more accuracy. If a bull's mates were tested under environment distinctly different from the average environment under which his daughters were tested, use of the mates' records could actually introduce bigger errors than it would remove. Doubtless that happens in some individual cases, but if the environmental effects on mates are correlated with those on their daughters more closely than 0.25, subtracting the mates' average from the daughters' average will make the error less than it would be if the mates' records were not used. That using the mates' records generally corrects the proof enough to be worth while is not conceded by all *(17, 26, 42, 56, 59)*.

To use only the daughters who are out of tested dams wastes the evidence

which is in the records of daughters whose dams were never tested. Most of this information can be had by assuming that the untested dams would have averaged the same as the tested ones, or the same as the contemporary average of the herd in which the daughters were tested.

Since the daughter average is generally about halfway[3] between the breed average and the average of the mates *(56)*, the optimum use of the mates' records is to give them only about half as much weight negatively as is given positively to the records of the daughters. This is accomplished merely by adding the daughter-dam difference to the daughters' average to form the "intermediate" or "equal parent" or "modified Mount Hope" index *(42)*. If this intermediate index is then coded by adding half of it to half of the average production of the breed, it becomes Rice's "regression" index. This coding leaves the accuracy of the index for comparing the sires exactly the same but reduces the scale of variation by putting any two sires just half as far apart on the "regression index" as they were on the "equal parent index."

4. *Using the herd average* to correct for herd-to-herd environmental differences has been advocated *(34, 50, 59)* but has not become used widely. The method presents several difficulties, both in principle and in practice, such as how the herd average should be computed, what cows should be considered contemporary, and what weight should be given to the herd average *(49)*. Measuring a cow's ability by the difference between her records and the herd average is an attempt to correct for differences in environment from herd to herd and (where only her contemporaries are used in the herd average with which each cow is compared) for differences in environment from year to year. Much the same thing as comparing the daughters with their dams is done except that the latter tends to correct also for differences between the average breeding values of the mates of various sires, is based on smaller numbers *(49)*, and is more vulnerable to environmental changes from year to year since daughter and dam usually make their records in different years.

To rate a cow entirely by the difference between her own production and the average of her contemporary herd mates is to assume implicitly that all herds average alike genetically. This is too extreme. Differences in environment are likely to be the major cause of differences between herd averages *(59)*, but herds will also differ at least a little in their genetic composition. If one could know what portion is genetic in the differences between herd averages, he could pay the proper average amount of attention to the herd average by considering each herd as a "family" *(43, 49)*.

Where management varies little between neighboring farms but the environment varies much from one locality to another, a group of similarly managed herds in the same locality might be treated as a single herd *(34)*. The practice

[3] That the correlation between mates' average and daughters' average actually is close to the convenient one-half is more of a coincidence than a fundamental biological law. This correlation contains something from genic similarities between daughter and dam but much more from the general tendency of daughter and dam to be tested in the same herd, i.e., from correlations between the herd environments of daughter and dam *(42)*.

in parts of Switzerland of proving sires by comparing the production of their daughters with the average production of all cows in the same testing association *(20)* is such an effort to correct automatically for local differences in climate, altitude, quality of pasture, etc.

Recently the Milk Marketing Board in Britain *(18)* has undertaken to prove bulls by comparing the daughters with their contemporaries in the same herd, plus an allowance for differences between herd averages which implies that the causes of differences between herd averages within breeds are one-fifth genetic and four-fifths environmental. In each herd the difference between the average of the sire's n_1 daughters and their n_2 contemporaries is given a weight of $\frac{n_1 n_2}{n_1 + n_2}$ in combining the differences from various herds into a single figure for that sire *(59)*.

At present the commonest policies concerning the herd average are to omit it entirely or merely to list it and let the observer make what use of it he will.

5. *The records of brothers and sisters* contain some information about an animal's breeding value. These records are not ordinarily included in any formal program for "proving" sires or dams. They are really a progeny test of the animal's sire or dam and are only a "sib test" of the animal itself. The common advice to use the son of a proved sire, if a proved sire himself cannot be had, is simply advice to use a bull whose paternal sisters are unusually good.

The sib test is less accurate than progeny test based on the same numbers and under the same conditions, although it is usually more than half as accurate. The sib test is less accurate because chance in Mendelian segregation will permit an individual animal to be considerably better or worse than is typical of its parents' breeding values. Thus, predicting an animal from its sibs cannot be expected to be perfectly accurate, even in the impossibly extreme case in which its sib test is so perfect that its parents' breeding values are known exactly.

The sib test usually becomes available about two years before any daughter completes a record. The sib test is often the best basis, other than the records of his dam and grandams, for making any decisions about a bull during the two or more years before he becomes proved. Decisions which must be made still earlier, such as those before he even has any sisters tested, may have to be based largely on sib tests of his parents, i.e., on the progeny tests of his grandparents. Something like 75% of all bulls born will be first, second, or third calves of their dams. Few of these will have even one maternal sister with a complete record before they are 2 years old. Most of them should begin to have paternal sisters with completed records by the time they are 3 years old if they were born in herds which test regularly. A few which were born when their sires were already old may have fairly complete paternal sib tests even when they are born.

Only a small fraction of the purebred bulls born can ever be progeny tested, since as many bulls as heifers are born and the proving of one bull requires mating him to at least 15 or 20 cows for 1 year. Hence, it is important to choose as accurately as possible which of the young bulls are to be proved.

Accuracy would be maximum if a sire's "proof" included his sibs' records

and all other pertinent information, each item weighted according to its usefulness in that combination. That becomes a selection index which varies with the kinds and amount of information present and can easily become too complicated to include in a formal program for proving sires. The construction and validity of selection indexes are outside the scope of this paper *(28, 29, 36)*. Some simple approximation to a selection index seems useful for proving dams and might include attention to their sibs. An old bull can have so many daughters who tell so much about him that information about his ancestors and collateral relatives would increase only slightly the accuracy of an appraisal of his breeding value, but a young bull or a cow cannot have enough daughters to make this information about sibs and parents unimportant.

6. *Own phenotype* indicates an animal's breeding value distinctly better than one offspring would because chance at Mendelian segregation and the uncertainty about the kind of inheritance received from the other parent do not intervene to dilute the relation between an individual's phenotype and its own breeding value, as they dilute the relation between its breeding value and the phenotype of an offspring. Under most circumstances, more than four offspring are required before the progeny test becomes a more accurate indicator of breeding value than own performance *(41)*, but the number depends on heritability of the character, the amount and kind of prior selection, and the correlation between the offspring for reasons other than that they have this one parent in common. If the character is sex-limited, as milk and fat production are, own phenotype is, of course, not available to help in proving animals of the sex which cannot express the character. A simple way to use own performance in proving dams is to include it in the progeny average with a weight of about three; that is, to proceed as if the cow has three more offspring than she actually does and as if these three all had the same production as this cow does. Three is an approximation to the correct weighting which will vary with heritability and with other circumstances but can hardly ever be less than 2.5 and might even exceed 5. Similar use of own phenotype for bulls may be worth considering when proving for type those who have only a few daughters.

7. *Special aspects of proving dams* which differ from those of proving sires hinge around these differences: *(a)* a dam can have only a few tested daughters and will be at least 7 years old by the time as many as three of them have records, whereas a bull can have many daughters and may be well proven by the time he is 6 years old; *(b)* a dam can have production records of her own whereas a sire cannot; and *(c)* the records of the daughters can be compared with the records of their dams for proving a sire, but for proving a dam, the records of her daughters cannot be compared with any actual records of their own sires. Daughters could, however, be compared with their contemporary herd averages in the proving of dams just as well as in the proving of sires.

Current proposals *(52)* for proving cows envisage using only the cow's own records, the records of her daughters, and the average record of the daughters of each of her proven sons. It is currently proposed not to call a cow "proven" unless she has had at least three tested offspring. Using also the records of the

cow's dam and of the cow's sibs, of which she would usually have several by the time she has tested daughters, would increase the accuracy of the "proof" although perhaps not enough to be worth the extra computations.

OFFICIALLY SPONSORED PROCEDURES FOR PROVING SIRES AND DAMS

The breed associations for a long time have given various designations, such as gold and silver medals, to sires with a minimum number of daughters attaining a certain level of production in the official testing systems. In a limited sense this might be called a method of proving sires, but the selection of the data introduces so much uncertainty that such designations really tell little except that the sire did have more than the usual number of daughters who produced more than the usual amount.

Current practices in computing "proof" are diverse. In some plans only the daughter average is used with no reference to the production of their dams. In that case, the minimum number of daughters required is usually ten. In plans which emphasize the difference between daughters and dams, the production of daughters out of untested dams may be stated also. Sometimes the records of daughters and dams are merely presented for the breeder to use or interpret as he will. If they are combined into an index, the most common practices are to use the excess of daughters over dams or to add that to the daughter's average (equal-parent or intermediate index), or to average this index with the breed average (Rice's "regression index") so as to reduce the variation of the index. No breed associations have yet incorporated a sliding scale for expressing in the proof the extra confidence which comes with larger numbers of daughters, although that feature is included in Britain in the Milk Marketing Board's "relative genetic values" *(18)*. One common step in this direction is to quote preliminary or "pre-proof" when the number of daughters is still small (usually 5 to 9 inclusive) and to require a larger number (usually at least 10) for definitely recognized proof *(3, 62, 63)*.

In Denmark the cattle breeding societies in 1945 established special stations for progeny testing the sires which are used widely in artificial breeding *(26)*. Between 14 and 20 of the oldest daughters of the bull are leased for a year and brought to the testing stations September 1, just before their first calving. There they are fed and milked under standardized conditions for an average of about 10 months. Each station tests the daughters of two or three bulls at a time. The aims of these testing stations are to reduce the environmentally caused variations, to shift the biased effects to random ones, and to have enough daughters that little error from the random sources remains in the averages. Dams' records are not used because rarely do more than two heifers come from the same farm and the heifers will be from a nearly random sample of dams in that district. This plan has already aroused keen interest in breeding and management practices, but whether it will improve the accuracy of sire selections enough to be worth the expense and effort remains to be seen.

Several associations in the United States have plans for using sire proof in

pedigrees in order to call attention to the young bulls whose sires, dams, and grandparents have unusually good proof and which are, therefore, presumably the best risks for trying as sires *(3)*. The most comprehensive is the Jersey "Star Bull Plan" in which stars are awarded to a young bull when the merit of his relatives is above established requirements *(2)*. This is a kind of selection index in which the bull is estimated farther and farther above the average of the breed as more and better information about him is available. Stars are not taken away from a bull when the information indicates him to be poorer than the breed standards. This feature keeps the plan from publicizing the bulls which presumably should be culled, but it is an incentive to test since the owner does not risk getting harmful publicity.

The association in Friesland in the Netherlands recognizes bulls as "Preferente Stieren" when they and their progeny meet certain standards *(55)*. A graphic daughter-dam comparison is shown for each such bull. For percentage fat he is also assigned a Mendelian formula which postulates four pairs of genes with nearly equal effects. It serves as a sire index having a range of nine classes and it gives a hint about uniformity of the progeny *(32)*.

In Switzerland *(20)* the Brown Swiss records may be corrected for altitude and for hours the cows worked at draft. Since the herds are small and many bulls are used cooperatively, the records of the bull's daughters are compared with the average of the local testing association rather than with the average of their own dams.

In New Zealand the Dairy Board issues "Sire Surveys" which compare the records of the bull's daughters with the mature cow average of the same herd in the same year *(62)*. The mature cow average is first converted into an "expectancy" figure which is the average of all daughters in herds in which the dams produced at the same level as these dams. Then the bull is considered proven good or bad according to how much his daughters are above or below the expectancy figure for dams with that average. A sire survey is "preliminary" when at least ten of the bull's daughters have one lactation, becomes "intermediate" when eight of these daughters have completed a second lactation, and is "final" when at least six of these daughters have completed three lactations.

Several have proposed to express each record as a percentage of the herd average, or of the breed average, or of the average of records made at the same age—perhaps a moving average extending over 5 or 10 years—and then to use these percentages as measures of a cow's production or for sire proof *(9, 34, 35, 50)*.

MISCELLANEOUS

Heritability of intraherd differences between single 305-day lactation fat records in HIR or DHIA data seems generally to be of the order of 0.2, although a few estimates have been lower than 0.15 or higher than 0.25 *(31, 54)*. If 0.2 is correct for heritability and the standard deviation is 80 lb., the genic standard deviation would be about 35 lb. About one bull in 40 would be expected to raise by that much or more the production of his daughters out of average cows,

whereas only about one in 500 would raise them by more than 50 lb. Questions about normality of distribution of genic values and about whether the heritability fraction is known correctly should prevent extreme reliance on these figures, but they indicate how the rate of improvement is limited by the amount of genic variation present at a given moment as well as by inaccuracies in estimating which animals do have the highest breeding values. The error in sire proof as now conducted must be large since in many cases a bull's daughters do average more than 50 lb. above their dams. The major reason in most cases is that the environment for daughters was better than that for mates although, of course, sampling errors play some part.

The rate of improvement reasonably possible for milk production in a whole breed has been estimated *(54)* as about 1% per year under natural service without using progeny tests or sib tests and about 2% per year with the optimum use of those and artificial insemination *(58)*. In either case achieving this much would require devoting all the selection efforts to milk production without diverting any attention to type or other characters. These estimates rest on several imperfectly known parameters, such as heritability, replacement rates, and calving intervals, but they seem at least roughly accurate.

Inbreeding has several effects, mostly minor, on the proving of sires and dams. If the sire is inbred, the part played by Mendelian sampling in determining what he will transmit to each offspring is reduced. This shifts part of r into s and thus makes the proof more accurate. If the daughters are inbred, the breeding values of sires and dams are correlated. This also makes the proof more accurate. For example, proving a non-inbred bull by his daughters out of his half sisters makes the s about 1.125 times as large as if his daughters had been out of unrelated mates. However, the inbreeding also shifts parts of the dominance and epistasis from r to b.

Inbreeding is especially useful to find whether a sire carries undesirable recessive genes, such as lethals. The most efficient way of testing whether a sire carries a particular recessive gene is to mate him to several cows who are homozygous for the recessive gene. The next most efficient way is to mate him to several known heterozygotes *(6, 7)*. Inbreeding is the next most efficient way, but unlike the other methods, the inbreeding tests for all kinds of recessives at once whether they are suspected or not. If the sire really is a carrier, about one-eighth of his calves out of his own daughters are expected to be recessives. Among heterozygous sires each tested on n of his own daughters, only $(7/8)^n$ are expected to escape detection. Such reasons led Wriedt *(66)* to recommend that all dairy bulls thought worth using should be bred as soon as possible to at least 15 of their own daughters in order to learn early whether a sire carried any harmful recessive genes in heterozygous condition. Stopping the further use of the carrier sire and any use of his sons would keep such recessive genes from spreading widely throughout the breed. Wriedt's proposal has not been followed widely anywhere but may meet more approval now that artificial insemination makes it possible to spread the genes of one sire widely. Some allowance for the usual depressing effects of inbreeding on characters such as

milk and fat production should be made when comparing the inbred progeny of one sire with the non-inbred progeny of other sires.

If *specific combining ability* (including both that caused by dominance and that caused by epistasis) is important, a sire's proof on one kind of mates may be distinctly different from his proof on another kind of mates. In that case, the kind of mates chosen for proving him would be important. This would reach its extreme in breeding plans aimed at utilizing overdominance. In general, the sire would be tested on the kind of mates on which he or his descendants are intended to be used.

If dominance of the favorable genes is an important obstacle to accurate selections but there is no overdominance—or if threshold effects of the "margin of safety" kind are important—the proof might be more accurate if extremely low producers were chosen as mates *(64)*. Little is known about whether differences in specific combining ability are important, much less about whether the differences which do exist are caused mainly by dominance, overdominance, or by epistasis. Therefore, little is certain about how much, if anything, could be gained by using special tester stocks for proving sires.

If *interactions between heredity and environment* are important, a different kind of cattle should be bred for each climate, system of management, or ecological niche large enough and different enough from the others to make that worth while. Then it would be especially important that the daughters and their dams be tested under the same conditions as those under which their descendants are intended to be used. The scanty data on the subject make it seem likely that these interactions are small and unimportant when animals belonging to the same breed and used in a moderately uniform region, climate, or system of management are compared. These interactions cannot be neglected so safely when widely different kinds of cattle for world-wide use in a wide variety of climates and systems of management are being compared.

Other characters than milk and fat production could be proved according to the same general principles, but the practical applications may differ widely for reasons such as the following. The character may show at a younger age, as size and much of type do, or at a still older age as longevity and broken udders do. It may show in both sexes, although correction may need to be made for its possibly different expression in males and females. Its heritability may be higher or lower. Environmental effects, such as those from differences between herds, may be more important or less important than they are for milk and fat production. Corrections for such effects may be made more effectively, or less so. The practical importance of the character may be too small to justify much attention, since each bit of attention to other things detracts from the already small amount of freedom to select for important things such as health, milk, and fat production.

Sex-linkage makes a daughter a slightly better indicator of her sire's transmitting ability than she is of her dam's. The effect is as if the sire were completely homozygous for his sex-linked genes while his mates are not. Sex-linkage would also make a bull's maternal relatives better indicators of his breeding

value than his paternal relatives, but it would make a cow's paternal relatives better indicators of her breeding value than her maternal relatives. These differences seem too trivial to be worth attention because only one chromosome pair out of the 30 in cattle carries sex-linked genes. Some have thought it worth attention *(46, 60)*, but the facts they interpreted as suggesting sex-linkage could have resulted from herd-to-herd environmental differences being larger between some kinds of relatives, such as cousins through a paternal grandsire, than between other kinds, such as cousins through a maternal grandsire.

Cow families have received much more mention in the writings on applied animal breeding than their importance justifies *(33)*. So far as the family consists of a cow and her own daughters, this is a case of proving a dam and has real utility. Such proof can be useful in deciding whether the cow's daughters and sons and their offspring are to be used more extensively or less extensively, even when it comes too late for deciding what to do with the cow herself. When the family is extended to include the granddaughters of the cow, the small amount those add to the information about the cow's transmitting ability runs considerable risk of being distorted by environmental changes which have occurred in the longer time interval which has elapsed. Many of the granddaughters are only cousins to each other and through their sires are more closely related to the cows in other "cow families" than to the average member of their own family. Little but self-deception is accomplished by extending the cow family to descendants more remote than granddaughters.

REFERENCES

(1) Allen, N. N. A Standard for Evaluation of Dairy Sires Proved in Dairy Herd Improvement Associations. *J. Dairy Sci.*, **27**: 835. 1944.

(2) American Jersey Cattle Club. Jersey Star Bulls. Leaflet. 1950.

(3) Ayrshire Digest. Regression Index Is Officially Adopted. *Ayrshire Digest*, **31** (1): 7. 1945.

(4) Bayley, N. D., and Heizer, E. E. Herd Data Measures of the Effect of Certain Environmental Influences on Dairy Cattle Production. *J. Dairy Sci.*, **35**: 540. 1952.

(5) Berge, S. Avkomsundersøkelser. *Norsk. Landbruk.*, **23/24**: 139. 1944.

(6) ———. Om de Forskjellige Måter til a Påvise Heterozygoti for Resessive Egenskaper Når Dominansen Er Fullstendig. *Nord. Jordbrugsforsk.* **3-4A**: 97. 1934.

(7) ———. The Probability of Homozygosity. *J. Heredity*, **44**: 217. 1953.

(8) Berry, J. C. Reliability of Averages of Different Numbers of Lactation Records for Comparing Dairy Cows. *J. Dairy Sci.*, **28**: 355. 1945.

(9) Berry, J. C. Evaluating Dairy Sires. *Holstein-Friesian World*, **49** (4): 321. 1952.

(10) Berry, J. C., and Lush, J. L. High Records Contrasted with Unselected Records and Average Records as a Basis for Selecting Cows. *J. Dairy Sci.*, **22**: 607. 1939.

(11) Bonnier, G. The Sire Index. *Acta Agr. Suec.*, **1**: 321. 1946.

(12) Braude, R. The Progeny Testing of Dairy Bulls. *Emp. J. Exptl. Agr.*, **16**: 90. 1948.

(13) Copeland, L. The Use of Records in Evaluating the Inheritance of Cows and in the Proving of Bulls. *J. Dairy Sci.*, **21**: 651. 1938.

(14) Davidson, F. A. Measuring the Breeding Value of Dairy Sires by the Records of Their First Few Advanced Registry Daughters. Ill. Agr. Expt. Sta., *Bull.* 270. 1925.

(15) Dickerson, G. E. Estimates of Producing Ability in Dairy Cattle. *J. Agr. Research*, **61**: 561. 1940.

(16) DICKERSON, G. E., AND HAZEL, L. N. Effectiveness of Selection on Progeny Performance as a Supplement to Earlier Culling in Livestock. *J. Agr. Research*, 69: 459. 1944.

(17) EDWARDS, J. The Progeny Test as a Method of Evaluating the Dairy Sire. *J. Agr. Sci.*, 22: 811. 1932.

(18) ———. A Method of Evaluating Progeny Test Results. *Report No. 4 of the Production Division, Milk Marketing Board*, Thames Ditton, Surrey, England. 1953.

(19) ELDRIDGE, F. E., AND SALISBURY, G. W. The Relation of Pedigree Promise to Performance of Proved Holstein-Friesian Bulls. *J. Dairy Sci.*, 32: 841. 1949.

(20) ENGELER, W. Die Leistungsverbessernden Stiere in der Schweizerischen Braunviehzucht. *Schweiz. Stammzuchtbuch Commission für Braunvieh*. Luzern. 1934.

(21) GAUNYA, W. S., AND BARTLETT, J. W. A Review of the Breeding Methods Used to Increase Production in Dairy Cattle. Purebred Dairy Cattle Association, Mimeo. 1948.

(22) GOODALE, H. D. A Sire's Breeding Index with Special Reference to Milk Production. *Am. Naturalist*, 61: 539. 1927.

(23) GOWEN, J. W. Genetic Criteria of Breeding Worth in Dairy Sires. *Intern. Dairy Congr.*, English Edition (Sec. 1): 19. 1931.

(24) GRAVES, R. R. Best Records vs. the Average of All Records for the Evaluation of a Sire's Inheritance for Level of Production. *J. Dairy Sci.*, 30: 21. 1947.

(25) GRAVES, R. R., AND FOHRMAN, M. H. Superior Germ Plasm in Dairy Herds. *Yearbook of Agriculture*, USDA p. 997. 1936.

(26) HANSEN, K. Afkomsprøver med tyre. VIII. Agr. Expt. Sta. of Denmark, *Bull.* 270. 1954.

(27) HANSSON, N. Kan Man Med Ekonomisk Fördel Höja Meddelfetthalten i den Af Vara Nötkreatursstammar Och Raser Lämnade Mjölken? Centralanstalten För Försoksväsendet Pa Jordbruksomradet. *Bull.* 78. 1913.

(28) HARVEY, W. R. Genetic Variation and Covariation in Type and Butterfat Among Jersey Cattle. Unpublished Ph.D. thesis, Iowa State College Library, Ames. 1949.

(29) HAZEL, L. N. The Genetic Basis for Constructing Selection Indexes. *Genetics*, 28: 476. 1943.

(30) HYATT, G., JR., TYLER, W. J., AND HENDERSON, H. O. More Milk Through Better Breeding as Shown by Research at West Va. University. West Va. Agr. Expt. Sta., *Bull.* 339. 1949.

(31) JOHANSSON, I. The Heritability of Milk and Butterfat Yield. *Animal Breeding Abstr.*, 18: 1. 1950.

(32) JOHNSON, L. E. The Importance of Uniformity of Get in Evaluating a Sire. *J. Dairy Sci.*, 28: 109. 1945.

(33) KÖRPRICH, H. Die Bedeutung der Kuhfamilien für die praktische Zuchtarbeit und die Möglichkeit ihrer Bewertung. *Züchtungskunde*, 20: 167. 1948.

(34) KRÜGER, L. Die Bestimmung der Milchleistungsfähigkeit. *Züchtungskunde*, 13: 49. 1938.

(35) LAMASTER, J. P. Production Factors. *Guernsey Breeders' J.*, 41: 304. 1932.

(36) LEGATES, J. E., AND LUSH, J. L. A Selection Index for Fat Production in Dairy Cattle, Utilizing the Fat Yields of the Cow and Her Close Relatives. *J. Dairy Sci.*, 37: 744. 1954.

(37) LONKA, T. Sonnien Jälkeläisarvostelusta. *Valtion Maatalouskoetoiminnan Julkaisuja*, 127. (English Summary). 1946. *(Animal Breeding Abstr.*, 15: 105. 1947).

(38) LÖRTSCHER, H. Variationsstatistische Untersuchungen an Leistungserhebungen in einer British-Friesian Herde. *Z. Züchtungsbiol.*, Reihe B. 39: 257. 1937.

(39) LUSH, J. L. The Number of Daughters Necessary to Prove a Sire. *J. Dairy Sci.*, 14: 209. 1931.

(40) ———. The Bull Index in the Light of Modern Genetics. *J. Dairy Sci.*, 16: 501. 1933.

(41) ———. Progeny Test and Individual Performance as Indicators of an Animal's Breeding Value. *J. Dairy Sci.*, 18: 1. 1935.

(42) ———. The Optimum Emphasis on Dams' Record when Proving Dairy Sires. *J. Dairy Sci.*, **27**: 937. 1944.

(43) ———. Family Merit and Individual Merit as Bases for Selection. *Am. Naturalist*, **81**: 241 and 362. 1947.

(44) ———, Norton, H. W., III, and Arnold F. Effects Which Selection of Dams May Have on Sires Indexes. *J. Dairy Sci.*, **24**: 695. 1941.

(45) ———, and Schultz, E. N. Pedigree Promise and Progeny Test Among Sires Proved in Iowa Cow Testing Associations. *J. Dairy Sci.*, **21**: 421. 1938.

(46) Madsen, K. Inheritance of Milking Capacity. *Nature*, **129**: 165. 1932.

(47) Mason, I. L. Bull Progeny Testing in Denmark. *Proc. Brit. Soc. Animal Prod.*, p. 106. 1952.

(48) Matson, J. The Influence of Heterosis on the Progeny Tests. From Records of 32 years' Breeding Experience in 17 Herds of the Government of India's Dairy Farms. *Proc. N. Z. Soc. Animal Prod., 6th Ann. Conf.* p. 73. 1946.

(49) McGilliard, L. D. Usefulness of the Herd Average in Estimating Breeding Values of Dairy Cattle. Unpublished Ph.D. thesis, Iowa State College Library, Ames. 1952.

(50) Patow, C. von. Weitere Studien Über die Vererbung der Milchleistung beim Rinde. *Z. Tierzücht. Züchtungsbiol. B*, **17**: 3. 1930.

(51) Plum, Mogens. Causes of Differences in Butterfat Production of Cows in Iowa Cow Testing Associations. *J. Dairy Sci.*, **18**: 811. 1935.

(52) Purebred Dairy Cattle Association. Proving Sires and Dams. *Hoard's Dairyman*, **94**: 872. 1949.

(53) Putnam, D. N., Bowling, G. W., and Conklin, C. T. The Use of First Records vs. the Average of All Records in Dam-Daughter Comparisons when Proving Sires. *J. Dairy Sci.*, **26**: 967. 1943.

(54) Rendel, J. M., and Robertson, A. Estimation of Genetic Gain in Milk Yield by Selection in a Closed Herd of Dairy Cattle. *J. Genetics*, **50**: 1. 1950.

(55) Rengers, Baron E. V. Welderen. Preferente Stieren in Friesland 1910-1932. Leeuwarden Provinciale Commissie Voor de Veefokkerij in Friesland. (Abs., *Animal Breeding Abstr.*, **3**: 246).

(56) Rice, V. A. A New Method for Indexing Dairy Bulls. *J. Dairy Sci.*, **27**: 921. 1944.

(57) Robertson, A. Artificial Insemination and Livestock Improvement. *Advances in Genet.*, **6**: 451. 1954.

(58) Robertson, A., and Rendel, J. M. The Use of Progeny Testing with Artificial Insemination in Dairy Cattle. *J. Genet.*, **50**: 21. 1950.

(59) Robertson, A., and Rendel, J. M. The Performance of Heifers Got by Artificial Insemination. *J. Agr. Sci.*, **44**: 184. 1954.

(60) Smith, A. D. B. Genetics of Dairy Cattle. *J. Dairy Research*, **10**: 370. 1939.

(61) Taussig, S. The Problem of Proving Dairy Bulls. *Intern. Review Agr. Monthly Bull. Agr. Sci. and Practice*, **28**: 69T. 1937.

(62) Ward, A. H., and Castle, O. M. Extension and Investigational Work. *28th Ann. Report New Zealand Dairy Board.* 1952.

(63) Ward, A. H., and Campbell, J. T. The Evaluation of Dairy Sires in New Zealand. *Empire J. Exptl. Agr.*, **8**: 249. 1950.

(64) Warwick, B. L., and Copeland, O. C. A Dairy Sire Progeny Test. *J. Heredity*, **25**: 177. 1934.

(65) Washbon, W. E., and Tyler, W. J. The Number of Proved Sons Necessary to Evaluate the Transmitting Ability of a Dairy Sire. *J. Dairy Sci.*, **33**: 293. 1950.

(66) Wriedt, C. *Heredity in Livestock.* Macmillan Company, New York. 1930.

(67) Wright, S. On the Evaluation of Dairy Sires. *Proc. Am. Soc. Animal Prod.*, 24th Ann. Meeting, 71. 1931.

(68) Yapp, W. W. Inheritance a Factor in Breeding Failures. Purebred Dairy Cattle Association, *Mimeo.* 1948.

RELATIONSHIP OF SIZE OF GROWING CATTLE TO PASTURE INTAKE AND ITS USE AS AN INDEX OF PALATABILITY[1]

C. M. MARTIN,[2] W. F. BRANNON,[3] AND J. T. REID
Department of Animal Husbandry, Cornell University, Ithaca, New York

Very few studies have been made of the relationship between the dry matter intake of grazing animals and body size. Garrigus and Rusk *(2)* and Crampton and Purdy *(1)* found no significant correlation between these variables in studies with beef cattle. In experiments in which Raymond *(4)* found the amount of feces voided by sheep to reflect the dry matter intake, the yield of feces was almost entirely independent of the amount of available herbage but was positively correlated with body size. This indicated that the body size of grazing sheep is related to dry matter intake.

Since methods of measuring the herbage intake by grazing animals have been refined recently, the relationship of size of growing cattle to intake is given further attention in this report. An additional objective of this study is to determine whether the relationship between body size and dry matter intake might be used to derive an index of palatability.

EXPERIMENTAL PROCEDURE

The amounts of dry matter consumed by growing steers grazing four different pastures were studied in relation to body size. At intervals during the grazing season, dry matter intakes from Pastures I, II, and III were derived from indigestibility coefficients determined by the fecal-chromogen method *(5)* and total fecal outgo measured with fecal collection bags *(3)*. Intakes from Pasture IV were measured in the same manner except that Cr_2O_3 was used as an indicator of fecal output *(3)*. Corresponding body weight measurements were made during six or seven consecutive days just before and during the intake-measurement trials conducted with Pastures I, II, and III. The steers grazing Pasture IV were weighed at the same time of the day at weekly intervals throughout the 105-day grazing period.

Pastures I, II, and III were grazed continuously during the same 77-day period by groups of three Holstein steers each. The body weights of the nine animals ranged from 350 to 460 lb. initially to 460 to 600 lb. at the end of the experiment. In the subsequent year, Pasture IV was grazed continuously by two beef steers weighing 575 and 640 lb. at the beginning, and 700 and 780 lb. at the end, of the 105-day period.

Received for publication August 23, 1954.

[1] Portions of these data were presented by C. M. Martin and W. F. Brannon in theses to the Graduate School, Cornell University, in partial fulfillment of the requirements for the Ph.D. degree, 1952 and 1953, respectively.

[2] Present address: Alabama Polytechnic Institute, Auburn.

[3] Present address: U. S. Department of Interior, The Squaw-Butte Harney Grazing Research Station, Burns, Ore.

The intakes of herbage from each of Pastures I, II, and III were measured during five periods at intervals of approximately 17 days. The steers were allowed to graze only small areas of these pastures at a time. As a consequence, the herbage of each successive area grazed was somewhat more mature than that of the previous one, except in the case of the aftermath pastures. The amount of herbage consumed from Pasture IV was determined during eight periods which constituted the 105-day grazing season. An amount of Pasture IV equivalent to about 1¾ acres per steer was grazed continuously throughout the season. No feeds other than herbage were provided to the steers grazing any of the pastures.

The botanical composition varied considerably not only among pastures, but also within pastures at different stages of growth. Pasture I, studied at four successive stages of uninterrupted growth and one aftermath stage, consisted largely of timothy and Kentucky bluegrass. The first-growth herbage of Pasture II, studied at three successive stages, contained 20 to 30% legumes and 70 to 80% timothy and quack grass. Aftermath of this pasture, on which intake was measured at two stages, contained 60% alfalfa. Intake measurements were made at three successive stages of first growth and at two stages of aftermath growth of Pasture III. The first growth of this pasture consisted of 85 to 95% timothy and quack grass and a small amount of legumes. Legumes made up 65 to 70%, and timothy and quack grass made up 30 to 35%, of the aftermath of Pasture III. Pasture IV consisted of approximately 35% legumes and 65% nonlegumes.

The digestibility of the dry matter of the herbages grazed ranged from 54 to 76%, indicating that the quality of herbage consumed was quite variable. However, an abundance of herbage existed at all times, establishing an ad libitum feeding regime.

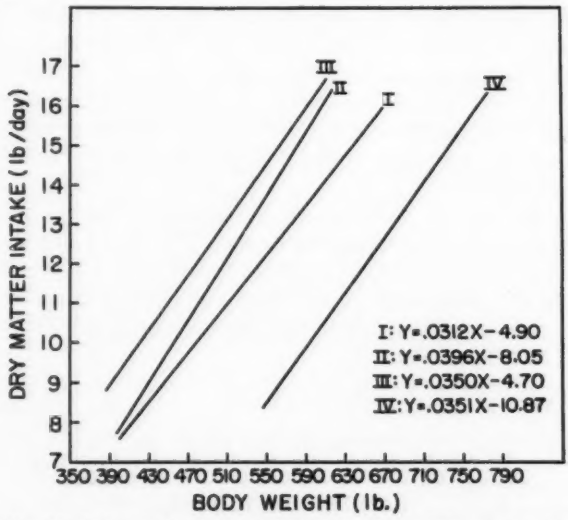


FIG. 1. Relationship between the body weight of growing cattle grazing four pastures and the daily intake of dry matter.

RESULTS AND DISCUSSION

Figure 1 demonstrates the relationship found between the body weight of growing cattle and the amount of dry matter consumed daily from each of the four pastures studied. As shown in Table 1, these variables are highly correlated (average within-pasture correlation, 0.83). The regression of body weight on dry matter intake was found to be highly significant for all pastures studied but, as indicated by the standard errors of estimate shown in Table 1, these equations afford only a relatively crude estimate of the dry matter intake from body weight.

These data were examined further in a covariance analysis *(6)*. It was found that the dry matter intakes among pastures were significantly different even after intakes were adjusted to a common body-weight basis. Therefore, size of animal does not account for the among-pasture differences in the intake. Presumably, most of the remaining differences are associated with the pastures.

The individual pasture regressions were not different from the average regression within pastures. Since the deviations from the among-pasture regression are not significantly different from those from the individual pasture regressions, the average within-pasture regression coefficient, 0.0353, becomes an expression of the relationship between daily dry matter intake and body weight changes.

Although these data are limited, it is indicated that, when sufficient herbage is available to satisfy the appetite for dry matter, the amount of dry matter consumed by growing cattle increases by 3.5 lb. per day for an increment of 100 lb. in body weight. The total intake per day, however, depends not only upon body size, but also upon the nature of the pasture. Therefore, the daily absolute intake by animals of varying size is not a satisfactory measure of palatability. Should the results of further study support these findings, it is suggested that an adjustment of the absolute dry matter intake of growing cattle in accordance with body weight would provide an adjusted intake which reflects the influence of pasture characteristics on dry matter intake devoid of the influence of body size. The adjusted intake, reflecting pasture qualities, then becomes an index of palatability.

In this study, the "adjusted" mean intakes shown in Table 1 were computed as follows: $\hat{Y} = Y_i - 0.0353\ (X_i - X)$, where: $\hat{Y}$ = "adjusted" mean dry matter intake (lb/day) for a particular pasture; Y_i = mean absolute dry matter intake (lb/day) for a particular pasture; X_i = mean body weight (lb.) of animals grazing a particular pasture and X = mean body weight (lb.) of animals grazing all pastures being compared.

The pastures examined in this experiment ranked, in order of decreasing palatability, III, II, I, and IV (Table 1). Because of the great need for an objective measure of the palatability of pastures, this approach is being suggested despite the relatively meager data. On the other hand, a wide range in kinds of herbage grazed is involved in this study. Growing beef cattle appeared to respond in the same way as growing dairy cattle, though years, kinds of pasture,

TABLE 1

Summary of data concerned with relationship of body weight of growing cattle to intake of pasture herbage

Pasture	Range in body weight	Average body weight	Average D.M. intake	Correlations between intake and size	Regression equations[a]	Standard error of estimates	Average body wt. gain	Adjusted D.M. intake
	(lb.)	*(lb.)*	*(lb/day)*			*(lb/day)*	*(lb/day)*	*(lb/day)*
I	389-581	481	10.12	0.79**	Y = 0.0312X − 4.90	1.37	1.6	11.74
II	391-593	482	11.07	0.85**	Y = 0.0396X − 8.05	1.39	1.7	12.64
III	353-565	472	11.84	0.89**	Y = 0.0350X − 4.70	1.20	1.7	13.77
IV	577-770	663	12.39	0.79**	Y = 0.0351X − 10.87	1.87	1.3	7.59
Average within pasture		527	11.37	0.83**	Y = 0.0353X − 7.22			11.37

[a] Y = Dry matter intake (lb/day); X = Body weight (lb.).

** Highly significant.

growth stage, and grazing methods are confounded. Since the body weights of the cattle used in these studies ranged from approximately 350 to 780 lb., there is no indication of the kind of response which might result from the use of larger and/or mature animals. It seems possible that lactating cows might be employed if an adjustment in intake is made for size of animal and level of production. Further studies are contemplated with a view to examining the influence of several variables upon the relationship between dry matter intake and body size of grazing cattle.

SUMMARY

An examination of the relationship of the body weight of growing cattle grazing four pastures to the dry matter intake revealed a highly significant correlation between these two variables. In addition, it was found that the dry matter intake increases by 3.5 lb. per day for a body weight increment of 100 lb., provided that sufficient herbage is available to satisfy the appetite. The relative constancy of the rate of change in intake with changing body weight suggests that an accounting for the influence of varying body weights upon the absolute dry matter intake provides an index of palatability. Although this approach to an objective measurement of palatability needs much more study, it would appear to be particularly useful in comparisons of different kinds of pastures and their responses to various treatments.

ACKNOWLEDGMENT

The authors appreciate the helpful suggestions made by C. R. Henderson.

REFERENCES

(1) Crampton, E. W., and Purdy, T. L. Pasture Studies. XXII. Dry Matter Defecation as an Index of Forage Intake by Grazing Steers. *Sci. Agr.*, **22**: 242. 1941.

(2) Garrigus, W. P., and Rusk, H. P. Some Effects of the Species and Stage of Maturity of Plants on the Forage Consumption of Grazing Steers of Various Weights. Ill. Agr. Expt. Sta., *Bull.* 454. 1939.

(3) Hardison, W. A., and Reid, J. T. Use of Indicators in the Measurement of the Dry Matter Intake of Grazing Animals. *J. Nutrition*, **51**: 35. 1953.

(4) Raymond, F. Evaluation of Herbage for Grazing. *Nature*, **161**: 937. 1948.

(5) Reid, J. T., Woolfolk, P. G., Hardison, W. A., Martin, C. M., Brundage, A. L., and Kaufmann, R. W. A Procedure for Measuring the Digestibility of Pasture Forage under Grazing Conditions. *J. Nutrition*, **46**: 255. 1952.

(6) Snedecor, G. W. *Statistical Methods.* 3rd ed. The Iowa State College Press, Ames. 1940.

FURTHER STUDIES ON THE PRODUCTION OF A HYPERKERATOSIS IN CALVES WITH TOPICALLY APPLIED BASE-OILS FOR USE IN LIVESTOCK SPRAYS[1]

W. G. HOEKSTRA, R. J. DICKE, AND P. H. PHILLIPS
Departments of Biochemistry and Entomology, University of Wisconsin, Madison

The control of flies in cattle management is a problem of significant economic and aesthetic importance. Application of insecticides directly on the animals is one of the common methods of fly control. Petroleum oils are frequently used as solvents or carriers of insect toxicants for these sprays, and indiscriminate use of such preparations may sometimes do more harm than good. Regan and Freeborn *(10)* studied the effect of flies and fly sprays on certain physiological processes of the dairy cow. They concluded that oils applied at the rate of 40 ml. or 80 ml. per cow per day reduced the amount of water passing osmotically through the skin, thus reducing the ability of the cow to regulate body temperature. This was highly important at environmental temperatures above 80° F. Under their conditions, the use of oils caused about twice the loss in milk production as that resulting from uncontrolled flies (20% reduction from the oil compared to a 10% reduction from flies). The work of Atkeson *et al. (1)* indicated that some base oils produced skin changes in cattle characterized by shedding of hair, scurfy flakes, excessive wrinkling, apparent thickening, and tenderness. Harshfield and Rehfeld *(6)* have reported a chronic dermatosis in cattle characterized by a thick crust of keratinized epithelium due to application of certain mange oils.

A previous publication described the production of a hyperkeratosis in calves with a topically applied oil-based insecticide carrier (mineral seal oil) *(7)*. This condition differed in several important respects from the disease designated as bovine hyperkeratosis, or X-disease, by Olafson *(9)*, which has since been shown to be related to the toxic effects of highly chlorinated naphthalenes *(2, 3, 5, 11, 12)*. However, the skin condition, although appearing much earlier, was similar if not identical to that encountered in chronic bovine hyperkeratosis.

The purpose of this report is to describe further experiments designed to study *(a)* the importance of dosage and frequency of application of mineral seal oil in producing hyperkeratosis, *(b)* the variations in ability of different oils to produce hyperkeratosis as related to source, degree of refinement, and their common chemical and physical specifications, and *(c)* the further characterization of this spray oil-produced hyperkeratosis as compared to bovine hyperkeratosis (X-disease) with special reference to the over-all effect on animals and the relationship to vitamin A metabolism. The ultimate objective of these studies was to ascertain the specific chemical and/or physical factors characteristic of hyper-

Received for publication September 11, 1954.

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station. Supported in part by the Standard Oil Co. (Indiana), Chicago.

keratosis-producing oils with the purpose of developing and specifying safe, yet economical, oils for spraying calves.

PROCEDURE AND RESULTS

Three separate experiments will be described.

Experiment I. This study was conducted from July through September of 1953 to investigate the importance of dosage and frequency of application of mineral seal oil used in previous investigations *(7, 8)* in the development of hyperkeratosis and the possible variation in hyperkeratosis-producing ability of certain oils. Twenty healthy Holstein-Friesian calves ranging in weight from 120 to 300 lb. were divided as equally as possible into ten lots of two animals each. An attempt was made to include a large and a small calf in each lot. After a pretreatment period of 10 days, the lots of calves were treated as follows:

Lot 1A — Untreated controls
Lot 1B — Oil No. 1 (mineral seal oil used in previous investigations) applied at the rate of 1/8 oz. per 100 lb. body weight per day
Lot 1C — Oil No. 1 applied at the rate of 1/3 oz. per 100 lb. body weight per day
Lot 1D — Oil No. 1 applied at the rate of 3/8 oz. per 100 lb. body weight every 3 days
Lot 1E — Oil No. 1 applied at the rate of 1 oz. per 100 lb. body weight every 3 days
Lot 1F — Oil No. 2 (highly refined white oil of light lubricating oil viscosity) applied at the rate of 1/3 oz. per 100 lb. body weight per day
Lot 1G — Oil No. 3 (similar to Oil No. 1 but treated with a different acid) applied at the rate of 1/3 oz. per 100 lb. body weight per day
Lot 1H — Oil No. 4 (similar to Oil No. 1 but highly refined) applied at the rate of 1/3 oz. per 100 lb. body weight per day
Lot 1I — Oil No. 5 (deodorized kerosene of low viscosity) applied at the rate of 1/8 oz. per 100 lb. body weight per day
Lot 1J — Oil No. 5 applied at the rate of 1/3 oz. per 100 lb. body weight per day

All of the oils used in the experiment were from a Mid-Continent crude petroleum source. Each lot of animals was penned separately. The oils were applied with hand sprayers over the back, sides, and underline of the calves from neck to rear as evenly as possible. The amount of oil (milliliters) applied to each lot was measured by difference in graduated cylinders, and the amount was balanced slightly from day to day to give the desired average dosages. The treatment period was 8 weeks. The calves were weighed each week, and blood plasma vitamin A concentrations were determined at intervals before and during the treatments. Observations for hyperkeratosis and other abnormalities were made throughout the experimental period. At the close of the experiment one

calf was taken from the control lot and one from the lot receiving the highest dosage of each of the oils for gross pathological examination, for histological examinations of skin, liver, and kidney, and for liver vitamin A determinations.

The results of Lots 1A through 1E demonstrated that Oil No. 1 (mineral seal oil) produced a pronounced hyperkeratosis when applied at the rate of ⅓ oz. per 100 lb. body weight per day (Lot 1C) or 1 oz. per 100 lb. body weight every 3 days (Lot 1E). The development of this condition has been described previously *(7)*. Control calves (Lot 1A) were normal in all respects. One-eighth oz. per 100 lb. body weight per day (Lot 1B) or ⅜ oz. per 100 lb. body weight every 3 days (Lot 1D) of this oil also produced hyperkeratosis but to a lesser degree. Figures 1 and 2 show the effect of the high daily dosage of the oil compared to the low daily dosage illustrated by Figures 3 and 4. The large and small calves

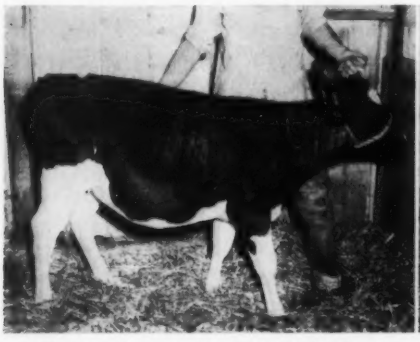


FIG. 1. Large calf of Lot 1C after 35 days of exposure to Oil No. 1 (mineral seal oil) topically applied at the rate of 1/3 oz/100 lb. body weight/day.

FIG. 2. Small calf of Lot 1C after 35 days of exposure to Oil No. 1 (mineral seal oil) topically applied at the rate of 1/3 oz/100 lb. body weight/day. Compare with large calf shown in Figure 1.

of each lot are shown to point out the apparently greater susceptibility of the smaller animals to the harmful effects of the oil. This relationship has been consistently observed throughout the study. The application of the same total quantity of oil applied at 3-day intervals rather than in daily applications (Lot 1B compared to Lot 1D, and Lot 1C compared to Lot 1E) resulted in about the same degree of hyperkeratosis. If any difference could be detected, it was in the direction of less effect from the larger applications of oil at greater intervals of time. The low daily dosage of Oil No. 1 represents the manufacturer's recommended application rates for adult cattle and the higher dosage, an excessive rate of application.

The results from the various oils used in Experiment I in the production of hyperkeratosis are summarized in the first part of Table 1. It was apparent that the oils behaved differently in their ability to produce hyperkeratosis. Oil No. 2, a highly refined, high-viscosity oil, produced no grossly detectable skin changes. A calf treated with this oil is shown in Figure 5. Oil No. 3 (mineral

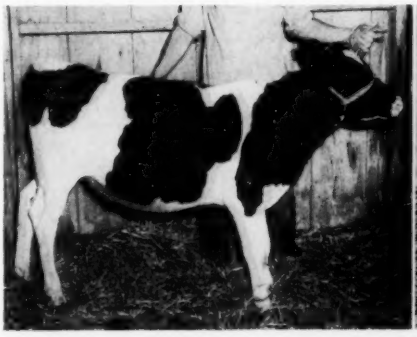


FIG. 3. Large calf of Lot 1B after 35 days of exposure to Oil No. 1 (mineral seal oil) topically applied at the rate of 1/8 oz/100 lb. body weight/day (manufacturer's recommendation for adult cattle). This calf shows only a slight degree of hyperkeratosis.

FIG. 4. Small calf of Lot 1B after 35 days of exposure to Oil No. 1 (mineral seal oil) topically applied at the rate of 1/8 oz/100 lb. body weight/day (manufacturer's recommendation for adult cattle). Compare the rather marked skin effect on this small calf with that of the animal shown in Figure 3.

seal oil treated with a different acid) and Oil No. 4 (a highly refined mineral seal oil) caused a hyperkeratosis comparable to that of Oil No. 1 (mineral seal oil). Oil No. 5 (low-viscosity, deodorized kerosene) at the high dosage level (Lot 1J), although not producing a hyperkeratosis, precipitated a rather marked desquamation, primarily along the neck. This condition is shown in Figure 6. At the lower application rate (Lot 1I) of Oil No. 5 only very slight scaling was observed.

Previous results indicated that this spray oil–produced hyperkeratosis was accompanied by a transitory, measurable, but not critical, depression in blood plasma vitamin A concentration *(7)*. Appetite and general well-being of calves were not seriously affected. The results in this experiment substantiate former observations. A summary of the important vitamin A data is presented in Table 2. The data from the calves have been classified into two groups, showing the calves which developed a hyperkeratosis, and those which showed no evidence of the disease. At 11 and 25 days post-treatment the calves showing hyperkeratosis had an average blood plasma vitamin A concentration about 3γ per 100 ml. of plasma lower than the calves showing no evidence of this skin condition. The average decline in the vitamin A level from 3 days pretreatment to 25 days post-treatment was $1.8 \pm 3.4\gamma$ per 100 ml. plasma for the non-hyperkeratosis group compared to $4.9 \pm 3.6\gamma$ per 100 ml. for the hyperkeratosis group. This difference only approached significance when analyzed by the t test ($t_{0.05} = 2.10$, $t_{0.10} = 1.73$, $t_{\text{observed}} = 1.85$). Thus, the plasma vitamin A decline accompanying this oil-produced hyperkeratosis, if present, is not critical. This apparent depression is transient, since at 49 days post-treatment the plasma vitamin A values were the same for both groups of calves even though the oils had been continually applied. The reason for the apparent plasma vitamin A depression

TABLE 1

Relative skin effects of the various petroleum oils studied in Experiments I and II (all oils applied at the rate of 1/3 oz/100 lb. body weight/day)

Exptl. lot	Oil No.	Brief description of oil[a]	Estimated relative degree of hyperkeratosis
1C	1	Mid-Continent mineral seal oil	++++
1F and 2B	2	Mid-Continent, highly refined, white oil in the light lubricating oil viscosity range	–
1G	3	Mid-Continent oil, similar to Oil No. 1, but treated with a different acid	++++
1H	4	Mid-Continent oil, similar to Oil No. 1 highly refined	++++
1J and 2C	5	Mid-Continent, low viscosity, deodorized kerosene	– scaling only
2E	6	Mid-Continent pale oil (not refined) of high viscosity	+ ?
2F	7	Mineral seal oil from an asphaltic base coastal crude	+ ?
2G	8	Highly paraffinic Pennsylvania oil in the mineral seal range	+++
2H	9	Untreated Mid-Continent oil in the mineral seal range	++++
2D	75% 2 25% 5	Proportioned from "safe" oils to give viscosity of mineral seal range	–
2I	50% 1 50% 5	Harmful oil diluted with a low viscosity "safe" oil	++

[a] See Table 3 for detailed inspections of these oils.

upon topical application of hyperkeratosis-producing oils is not understood. Liver vitamin A determinations at the termination of the experiment (62 days post-treatment) for three calves showing no hyperkeratosis (Lots 1A, 1F, 1J) averaged 15.3γ vitamin A per gram fresh liver compared to 15.0γ per gram liver for three calves showing severe skin changes (Lots 1C, 1G, 1H).

The calves developing hyperkeratosis gained an average of 92 lb. in body weight throughout the experimental period while those which showed no hyperkeratosis gained an average of 98 lb. It is readily apparent that no significant depression of weight gains resulted from the hyperkeratosis under the conditions of this experiment.

No consistent internal gross pathological changes were observed at the termination of the experiment in the representative animals treated with each oil. The results of the histological examinations will be discussed after Experiment II.

Experiment II. From the results of Experiment I it was evident that various oils differed greatly in their ability to produce hyperkeratosis. Experiment II was conducted in October and November, 1953, to study further the possible factors distinguishing dermatoxic oils from "safe" oils. The first experiment suggested that an intermediate viscosity range may be associated with toxicity regardless of refinement and the hyperkeratosis may be related to this purely

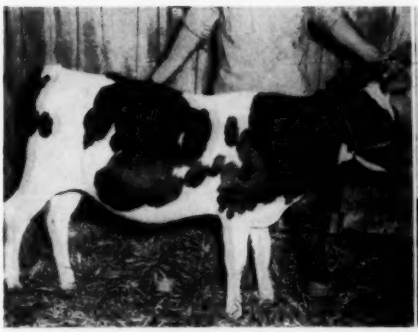


FIG. 5. Large calf of Lot 1F after 35 days of exposure to Oil No. 2 (highly refined white oil of relatively high viscosity) topically applied at the rate of 1/3 oz./100 lb. body weight/day. This calf, as well as the smaller calf of this lot, showed no detectable skin changes.

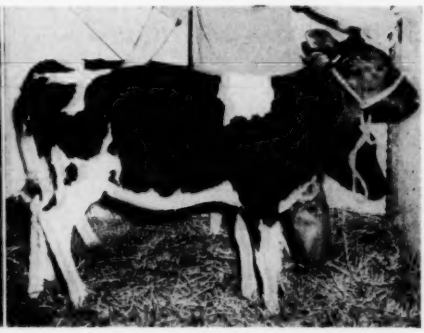


FIG. 6. Large calf of Lot 1I after 35 days of exposure to Oil No. 5 (deodorized kerosene of low viscosity) topically applied at the rate of 1/3 oz/100 lb. body weight/day. This lot developed desquamation (scaliness) but no hyperkeratosis.

physical factor. It was also of interest to study the relative effects of oils of different crude sources.

The procedure used was that described for Experiment I, except that blood plasma and liver vitamin A determinations were not made. All oils were applied at the rate of ⅓ oz. per 100 lb. body weight per day. A new series of 18 calves weighing from 120 to 370 lb. was obtained for nine lots treated as follows:

Lot 2A — Untreated control
Lot 2B — Oil No. 2 (see Experiment I)
Lot 2C — Oil No. 5 (see Experiment I)
Lot 2D — Mixture of 75% Oil No. 2 and 25% Oil No. 5 (proportioned to give the viscosity of Oils 1, 3, and 4 of Experiment I)
Lot 2E — Oil No. 6 (pale oil of high viscosity from Mid-Continent crude, not refined)
Lot 2F — Oil No. 7 (mineral seal oil from an asphaltic base, coastal crude)
Lot 2G — Oil No. 8 (highly paraffinic, Pennsylvania oil in the mineral seal range)
Lot 2H — Oil No. 9 (untreated Mid-Continent oil in the mineral seal range)
Lot 2I — Mixture of 50% Oil No. 1 and 50% Oil No. 5 (a dermatoxic oil combined with a low viscosity, nontoxic oil representing some base oils being used commercially).

The calves were treated for 5 weeks. At the termination of the experiment the calves were slaughtered and samples of skin, liver, and kidney were collected for histological studies.

A summary of the relative degrees of hyperkeratosis produced by these

TABLE 2

Summary of the blood plasma vitamin A data of Experiment I as affected by oil-produced hyperkeratosis

Description of group considered	Av. blood plasma vitamin A conc. (γ/100 ml.)				
	Pretreatment		Post-treatment		
	7/13/53 −10 days	7/20/53 −3 days	8/3/53 +11 days	8/17/53 + 25 days	9/10/53 +49 days
All calves not showing hyperkeratosis—includes eight calves of Lots 1A, 1F, 1I, 1J	14.4 ± 3.3[a]	14.8 ± 2.3	15.2 ± 4.5	13.0 ± 3.9	16.5 ± 3.6
All calves showing hyperkeratosis—includes 12 calves of Lots 1B, 1C, 1D, 1E, 1G, 1H	14.0 ± 5.3	14.8 ± 5.3	12.3 ± 4.0	9.8 ± 2.7	16.4 ± 4.2

[a] Mean ± std. deviation.

treatments is shown in Table 1. Lots 2A, 2B, 2C, and 2D exhibited no hyperkeratosis. Thus, when the high viscosity negative oil (No. 2) was mixed with the low viscosity negative oil (No. 5) to give a viscosity approximating that of toxic oils No. 1, 3, and 4, no hyperkeratosis was produced. Therefore, viscosity does not appear to be a criterion for the hyperkeratosis-producing ability of an oil. The smaller calves of each of Lots 2E and 2F died during the experiment, of respiratory infection apparently unrelated to treatment (11 and 18 days after spraying was begun). The evaluations of the results from Oils No. 6 and 7 are subject to question, since the larger calves remaining in the lots treated with these oils tended to show less skin effect than younger calves. When these remaining large calves were compared to the large calves of Lots 2G, 2H, and 2I, however, the degree of hyperkeratosis was definitely less marked in the former. The highly paraffinic Pennsylvania oil of the mineral seal range (No. 8 of Lot 2G) produced a pronounced hyperkeratosis like that of the unrefined Mid-Continent oil of the mineral seal range. When Oil No. 1 (harmful) was mixed with equal volumes of Oil No. 5 (safe) the effect in reducing the degree of hyperkeratosis was that expected from a dilution of the skin-damaging component. The marked reduction in viscosity by adding the deodorized kerosene (No. 5) did not eliminate the hyperkeratosis-producing ability of the mineral seal oil (No. 1).

Histological examinations of skin sections taken from the necks of the calves at slaughter revealed that no apparent skin damage was produced in Lots 2A, 2B, 2C, 2D, and 2F. Minor increase in epithelial tissue was seen in Lot 2E, and moderate to severe hyperkeratosis and epithelial cell increase were evident in all calves of Lots 2G, 2H, and 2I. The histological evaluations of the calves in this experiment and in Experiment I agreed well with evaluations of the degree of hyperkeratosis based on gross observation.

Some inflammatory reaction was seen in the livers and some glomerular damage in the kidneys of some of the calves, including the controls. The changes in these organs are those so often encountered in the general cattle population that they cannot be considered to be associated with the oil treatments. In no calves was general health significantly affected.

Experiment III. The purpose of this experiment was to determine whether oral administration of hyperkeratosis-producing oils would affect blood plasma vitamin A concentrations or would produce any symptoms of bovine hyperkeratosis. Three lots of two calves, each weighing about 350-400 lb., were used. Lot 1 served as an untreated control, Lot 2 received Oil No. 1, and Lot 3 received Oil No. 5. The oils were administered by capsule in the amounts of 5 ml. twice daily from July 4 to August 4, 1953, and 10 ml. twice daily from August 5 to September 10. The total dosage of oil was therefore 1,030 ml. given over a period of 67 days. This dosage was believed to represent more than could possibly be ingested by the calves from licking sprayed animals or pens. It apparently was not enough to inhibit the absorption of fat soluble components of the diet. Blood plasma vitamin A determinations were made on July 2, 13, and 27, on August 10, and on September 11. Blood plasma vitamin A values for Lot 2 and Lot 3 did not digress significantly from those of the control calves. These oil treatments had no effect on the general well being of the calves, and no symptoms of bovine hyperkeratosis were observed throughout the experimental period.

DISCUSSION

Results from the experiments described and previous studies emphasize the differences between X-disease or bovine hyperkeratosis and the petroleum oil-produced hyperkeratosis. Although the term hyperkeratosis is frequently used interchangeably with the term X-disease, it actually refers to only one of the symptoms of the X-disease syndrome. This is important in relation to topically applied mineral seal oils, since they produce a hyperkeratosis often indistinguishable from that of chronic X-disease without the other manifestations of that disease. Figures 7 and 8 show the similarity of hyperkeratosis produced by a petroleum oil and that by chronic chlorinated naphthalene poisoning.

It was previously reported that proliferative, ulcerative, and abrasive lesions developed on the oral mucosa and tongues of some of the calves sprayed with mineral seal oil *(7)*. These types of lesions have since been observed also in control calves, and their incidence on the various oil treatments appears not to be associated with the hyperkeratosis-producing ability of these oils. The characteristic internal pathology of bovine hyperkeratosis *(9)* was not observed in calves severely affected with oil-produced hyperkeratosis. From the investigations reported in this communication and from previous studies it can be said that except for very young calves, hyperkeratosis-producing oils caused little if any reduction in appetite or weight gains under reasonably good nutritional and environmental conditions. The plasma vitamin A drop was transient but not critical after application of the oils. At the dosages used it was impossible to elicit any symptoms of bovine hyperkeratosis by oral administration of a hyperkeratosis-producing oil, and plasma vitamin A values were not depressed. Chlorinated naphthalenes were not detected in the mineral seal oil, according to the color reaction given by these materials with dimethyl aniline, as described by Engel *et al. (4)*. The evidence indicates that the condition produced by petroleum oils is of entirely different origin from that of chlorinated naphthalene poisoning.

FIG. 7. Close-up of the oil-produced hyperkeratosis (Oil No. 1 applied at the rate of 1/3 oz/100 lb. body weight/day for 35 days).

FIG. 8. Close-up of the skin condition of chronic bovine hyperkeratosis (X-disease) caused by ingestion of feed contaminated with highly chlorinated naphthalene. Note the striking similarity to Figure 7.

The factor(s) responsible for the production of hyperkeratosis from certain petroleum oils has not been elucidated and may represent a chemical and/or a physical phenomenon. Regan and Freeborn *(10)* stated that burning of the skin (redness and sloughing) followed the use of oils of viscosity below 40 SSU (Seconds Saybolt Universal) irrespective of the unsulfonated residue and that an unsulfonated residue of 90% was dangerous in oils of viscosity higher than 65 SSU. From a study on 17 oils, Atkeson *et al. (1)* reported that viscosity was apparently not related to the toxicity of the oils (range from 32 to 86 SSU), but the unsulfonated residue was correlated with toxicity. The oils with an unsulfonated residue ranging between 92.5 and 100% included all but one of the most satisfactory oils. Our work to date indicates that neither viscosity nor unsulfonated residue as such is a reliable criterion for hyperkeratosis-producing ability of an oil. Although both of our "safe" oils had unsulfonated residues above 96.6%, there were two oils with unsulfonated residues of 97% and 96.4% that produced marked hyperkeratosis. One of the "safe" oils had a high viscosity and the other a very low viscosity, the dermatoxic oils having intermediate viscosities. However, when the two "safe" oils were mixed to give a viscosity in the range of the toxic oils, no hyperkeratosis resulted. The more volatile, low viscosity oil may, however, have evaporated from the surface of the calves rather readily, leaving only the higher viscosity oil. The harmful effects of oils are often correlated with their content of aromatic compounds. It is of interest that a highly paraffinic Pennsylvania oil produced a pronounced degree of skin damage, whereas a mineral seal oil from an asphaltic-base, coastal crude oil appeared to elicit less effect.

The fact remains that some oils in the range suitable for use as insecticide carriers produce no skin changes when applied to calves in excessive amounts

(⅓ oz. per 100 lb. body weight per day). As yet no criteria for specifying a "safe" oil have been found.

TABLE 3
Inspections of oils used

Specification	Oil No. 1	2	3	4	5	6	7	8	9	75% 2 25% 5	50% 1 50% 5
Gravity API	36.6	37.2	36.6	39.5	45.8	31.0	31.8	41.4	35.1		42.2
ASTM distillation ° F.											
Initial	510	540	515	510	358	536	503	514	486		360
10% rec.	538	570	532	535	388	577	517	535	529		396
20% rec.	548	588	544	550	398	595	525	543	546		416
30% rec.	558	600	554	562	406	610	531	547	556		436
40% rec.	566	614	564	571	414	622	541	554	566		457
50% rec.	575	629	572	581	420	636	549	557	578		482
60% rec.	584	643	583	590	427	649	560	563	590		515
70% rec.	594	661	596	602	435	666	571	567	603		556
80% rec.	608	683	611	616	445	686	589	576	620		590
90% rec.	626	716	636	638	459	719	620	591	645		630
Max.	665	736	690	674	497	740+	664	644	683		646
Vis. at 100° F. *(SSU)*	43	54.2	43.2	43.9	[a]	58.5	40.7	40.4	43.1	43.0	34.9
Kinematic vis. at 100° F. *(cs)*	5.2	8.65	5.3	5.9	1.65	—	—	—	—	—	—
Unsulfonated residue *(%)*	91.5	100	90.4	97.0	96.6	81.8	74.6	96.4	86.4		93.5
Estimated degree of hyperkeratosis-producing ability	++++	−	++++	++++	(scaling only) −	+?	+?	+++	++++	−	++

[a] The viscosity of Oil No. 5 is below the SSU scale where inaccuracies are considerable.

SUMMARY

Experiments were conducted to further investigate *(a)* the importance of dosage and frequency of application of a mineral seal oil in producing a hyperkeratosis in calves, *(b)* the variations in the hyperkeratosis-producing abilities of various oils, and *(c)* the relation of the condition produced by the oils to bovine hyperkeratosis (X-disease).

A pronounced hyperkeratosis developed in calves upon topical administration of ⅓ oz. of mineral seal oil per 100 lb. body weight per day. A dosage of 1 oz. of oil per 100 lb. body weight applied every 3 days resulted in a comparable degree of hyperkeratosis. A lower application rate of this oil as recommended by its manufacturer for adult cattle (⅛ oz. per 100 lb. body weight per day) also produced a hyperkeratosis, but to a much less degree.

Eleven different oil preparations differed greatly in their ability to cause skin changes. When topically applied at the rate of ⅓ oz. per 100 lb. body weight per day, two of these produced no detectable skin changes, one caused only desquamation with no hyperkeratosis, three produced intermediate degrees of hyperkeratosis, and five resulted in pronounced hyperkeratosis. The ability of an oil to produce hyperkeratosis as contrasted to a "safe" oil could not be explained by crude source, viscosity, unsulfonated residue, boiling range, or the

acid used in refinement. No chlorinated naphthalene could be detected in a hyperkeratosis-producing oil. As yet no chemical or physical criteria have been found for specifying a "safe" oil.

The hyperkeratosis produced in calves by these petroleum oils was indistinguishable from that of chronic bovine hyperkeratosis or X-disease. The skin condition, however, was the only symptom of bovine hyperkeratosis consistently produced by the oils. The oil-produced hyperkeratosis caused an apparent, noncritical, transient blood plasma vitamin A depression. Under good nutritional and environmental conditions the skin damage was not associated with any decrease in body weight gains when the oils were applied at the rate of ⅓ oz. per 100 lb. body weight per day for 50 days. Oral administration of a hyperkeratosis-producing oil in the amount of 5 ml. of oil twice a day for 31 days followed immediately by 10 ml. twice a day for 36 days resulted in no plasma vitamin A depression, and no symptoms of bovine hyperkeratosis (X-disease) developed.

ACKNOWLEDGMENTS

The authors wish to express their sincere appreciation to S. H. McNutt of the Veterinary Science Department for doing the histopathological phases of this study and for his advice during the investigation, and to the Standard Oil Company (Indiana) Research Department for furnishing oil samples, specifications, advice, and counsel.

REFERENCES

(1) Atkeson, F. W., Borgmann, A. R., Smith, R. C., and Shaw, A. O. Relative Effects of Several Base Oils Used in Livestock Sprays on the Skin of Cattle. *J. Econ. Entomol.*, **37**: 419. 1944.

(2) Bell, W. B. Further Studies on the Production of Bovine Hyperkeratosis by the Administration of a Lubricant. *Virginia J. Sci.*, **3**: 169. 1952.

(3) Bell, W. B. The Relative Toxicity of the Chlorinated Naphthalenes in Experimentally Produced Bovine Hyperkeratosis (X-disease). *Vet. Med.*, **48**: 135. 1953.

(4) Engel, R. W., Price, N. O., Linkous, W. N., and Bell, W. B. A Colorimetric Method for the Detection of Highly Chlorinated Naphthalenes. Va. Agr. Expt. Sta., *Tech. Bull.* 121. 1953.

(5) Hansel, W., Olafson, P., and McEntee, K. Bovine Hyperkeratosis: Studies on a German Wood Preservative. *Cornell Vet.*, **43**: 311. 1953.

(6) Harshfield, G. S., and Rehfeld, C. E. A Chronic Dermatosis of Cattle Due to Oil Applications. *J. Am. Vet. Med. Assoc.*, **112**: 446. 1948.

(7) Hoekstra, W. G., Dicke, R. J., and Phillips, P. H. Production of Hyperkeratosis in Calves with a Topically Applied Oil-Based Insecticide Carrier. *Am. J. Vet Research*, **15**: 47. 1954.

(8) Hoekstra, W. G., Dicke, R. J., and Phillips, P. H. The Advantage of Wettable Powder or Emulsion Formulations of Insecticides over Oil-Based Preparations on Calves. *J. Econ. Entomol.* (Submitted for publication.)

(9) Olafson, P. Hyperkeratosis (X-disease) of Cattle. *Cornell Vet.*, **37**: 279. 1947.

(10) Regan, W. M., and Freeborn, S. B. The Effect of Flies and Fly Sprays on Certain Physiological Processes of the Dairy Cow. *J. Dairy Sci.*, **19**: 11. 1936.

(11) Sikes, D., and Bridges, M. E. Experimental Production of Hyperkeratosis (X-disease) of Cattle with a Chlorinated Naphthalene. *Science*, **116**: 506. 1952.

(12) Sikes, D., Wise, J. C., and Bridges, M. E. The Experimental Production of "X-disease" (Hyperkeratosis) in Cattle with Chlorinated Naphthalenes and Petroleum Products. *J. Am. Vet. Med. Assoc.*, **121**: 337. 1952.

EFFECT OF ANTIOXIDANTS IN THE CONTROL OF OXIDIZED FLAVOR DEVELOPMENT IN STORED FROZEN CREAM. II. USE OF ETHYL HYDROCAFFEATE

A. J. GELPI, JR., E. W. BRYANT, AND L. L. RUSOFF
Department of Dairying, Louisiana Agricultural Experiment Station, Baton Rouge

It has been reported previously by Gelpi *et al. (5)* that Sustane (butyl hydroxyanisole), Tenox II (butylated hydroxyanisole + citric acid + propyl gallate in propylene glycol), Tenox BHA (butylated hydroxyanisole without synergists), and ethyl caffeate (an ester of caffeic acid) showed varying degrees of effectiveness in delaying or preventing the development of oxidized flavors in frozen cream. In these trials, in which cream was stored in both glass and metal containers, with and without added copper, the results obtained with ethyl caffeate indicated that this compound possesses some outstanding properties as an antioxidant. In the present study, ethyl hydrocaffeate, a hydrogenated form of ethyl caffeate, was used as an antioxidant. In view of the work done by several investigators *(2, 3, 4, 6)* on the use of ascorbic acid as a synergist with antioxidants in milk, the effect of adding ascorbic acid with and without the ethyl hydrocaffeate on the development of the oxidized flavor in cream was examined.

EXPERIMENTAL PROCEDURE

Cream of good quality produced by the Louisiana State University dairy herd was used in all trials. The cream was standardized to 40% fat, pasteurized at 160° F. for 30 minutes, and cooled immediately to 40° F.

A. *Ethyl hydrocaffeate.* After cooling, the cream was divided into four portions; one served as a control and the others were treated with the following concentrations of the antioxidant: 0.04%, 0.02%, and 0.004%, based on the weight of the fat. The antioxidant was dissolved in a minimum quantity of 95% ethanol, which amounted to less than 1 ml. After addition of the antioxidant, the cream samples were stirred for several minutes with a mechanical stirrer to insure complete dispersion. The cream samples were further divided into four portions, each of which was stored in glass containers and in friction-top tinned cans with and without 0.5 p.p.m. Cu from $CuSO_4$. Thus there were 16 lots, each containing seven identical samples. One sample from each lot was used for an organoleptic examination every 30 days for a period of 6 months and then again at the end of 12 months. The samples were stored at –10° F. and held at this temperature until examined for oxidized flavor development by two judges. The results of Trial A are shown in Tables 1 and 2.

B. *Ascorbic acid.* In this trial only tinned cans were used for storage of samples, since glass containers were considered impractical from a commercial standpoint. A control sample and cream treated with 20, 25, and 40 mg. per

Received for publication September 24, 1954.

TABLE 1

The antioxygenic effect of ethyl hydrocaffeate added to cream stored in glass jars and tinned cans—May, 1952–May, 1953

Sample	Glass jars							Tinned cans						
	Months							Months						
Conc. ethyl hydrocaffeate	1	2	3	4	5	6	12	1	2	3	4	5	6	12
(%)														
0 (control)	−	−	−	++	++	++	++	−	++	+++	+++	+++	+++	+++
0.04	−	−	−	−	−	−	−	−	−	−	−	−	−	−
0.02	−	−	−	−	−	−	−	−	−	−	−	−	+++	+++
0.004	−	−	−	−	−	−	−	−	−	++	++	++	++	++
0.04 + 0.5 p.p.m. Cu	−	−	−	−	−	−	−	−	−	−	−	−	−	−
0.02 + 0.5 p.p.m. Cu	−	−	−	−	−	−	−	−	+	+	+	+	+	+
0.004 + 0.5 p.p.m. Cu	−	+	+	+	+	+	+	−	+++	+++	+++	+++	+++	+++

− not oxidized.
+ slightly oxidized.
++ pronounced oxidation.
+++ very pronounced oxidation.

liter concentrations of ascorbic acid were used. After thorough agitation with a mechanical stirrer, each sample was divided into two portions, one receiving 0.5 p.p.m. of copper and the other receiving no copper. This series comprised eight lots, each containing seven identical samples. Samples were examined every 30 days and again in 12 months for the presence of an oxidized flavor.

C. *Ascorbic acid plus ethyl hydrocaffeate.* In this trial all samples were treated with the same concentrations of ethyl hydrocaffeate (0.04, 0.02, and 0.004%), with and without added copper, in tinned cans, as described under Section B. In addition, 20, 25, and 40 mg. per liter of ascorbic acid were added to each concentration of the ethyl hydrocaffeate. Samples were examined by the same judges.

RESULTS AND DISCUSSION

The effect of adding ethyl hydrocaffeate to cream in glass containers on the development of oxidized flavor while in frozen storage is shown in Table 1. In this trial all treated samples, with and without copper contamination, showed no development of the oxidized flavor for a 12-month period, with the exception of the one containing 0.004% of the antioxidant with added copper. Table 1 also shows the results on duplicates of samples used in the first trial but stored in friction-top tinned cans. These results indicate that metal containers definitely exert some influence on the development of oxidized flavors in cream during frozen storage. However, in order to simulate commercial conditions as nearly as possible, only tinned metal containers were used in the remaining trials.

TABLE 2

The antioxygenic effect of ascorbic acid added to cream stored in tinned cans February, 1953–February, 1954

Sample Conc. ascorbic acid	Months						
	1	2	3	4	5	6	12
(mg/1)							
0 (control)	−	−	−	−	−	−	+++
20	+	+	++	++	+++	+++	+++
25	−	−	+++	+++	+++	+++	+++
40	−	−	−	++	++	+++	+++
20 + 0.5 p.p.m. Cu	+	+	+	+	++	++	++
25 + 0.5 p.p.m. Cu	−	+	+	+	+	+	+
40 + 0.5 p.p.m. Cu	+	+	++	++	++	+++	+++

− not oxidized.
+ slightly oxidized.
++ pronounced oxidation.
+++ very pronounced oxidation.

Table 2 shows results of trials in which ascorbic acid alone was added to cream stored in tinned cans. It is interesting to note that the oxidized flavor developed more rapidly and more intensely in the ascorbic acid-treated samples than in the untreated control. Brown *et al. (3)* reported similar results when ascorbic acid was added to milk.

The results of trials using ethyl hydrocaffeate plus ascorbic acid without added copper in frozen cream stored in tinned cans are shown in Table 3. These data indicate definitely that the effectiveness of ascorbic acid as a synergist with ethyl hydrocaffeate is markedly increased even in copper-contaminated cream. No oxidized flavor was noted after 12 months of storage in the samples treated with 0.004% ethyl hydrocaffeate and 25 mg. ascorbic acid with added copper.

TABLE 3

The antioxygenic effect of various levels of ascorbic acid and ethyl hydrocaffeate added to cream stored in tinned cans with and without added copper February, 1953–February, 1954

Sample	Months in storage						
0.04% ethyl hydrocaffeate +	1	2	3	4	5	6	12
20 mg. ascorbic acid per l.	−	−	−	−	−	−	−
25 mg. ascorbic acid per l.	−	−	−	−	−	−	−
40 mg. ascorbic acid per l.	−	−	−	−	−	−	−
0.02% ethyl hydrocaffeate +	1	2	3	4	5	6	12
20 mg. ascorbic acid per l.	−	−	−	−	−	−	−
25 mg. ascorbic acid per l.	−	−	−	−	−	−	−
40 mg. ascorbic acid per l.	−	−	−	−	−	−	−
0.004% ethyl hydrocaffeate +	1	2	3	4	5	6	12
20 mg. ascorbic acid per l.	−	−	−	−	−	+++	+++
25 mg. ascorbic acid per l.	−	−	−	−	−	?	?
40 mg. ascorbic acid per l.	−	−	−	−	−	−	−
0.04% ethyl hydrocaffeate + 0.5 p.p.m. Cu +	1	2	3	4	5	6	12
20 mg. ascorbic acid per l.	−	−	−	−	−	−	−
25 mg. ascorbic acid per l.	−	−	−	−	−	−	−
40 mg. ascorbic acid per l.	−	−	−	−	−	−	−
0.02% ethyl hydrocaffeate + 0.5 p.p.m. Cu +	1	2	3	4	5	6	12
20 mg. ascorbic acid per l.	−	−	−	−	−	+	+
25 mg. ascorbic acid per l.	−	−	−	−	−	−	−
40 mg. ascorbic acid per l.	−	−	−	−	−	−	−
0.004% ethyl hydrocaffeate + 0.5 p.p.m. Cu +	1	2	3	4	5	6	12
20 mg. ascorbic acid per l.	−	−	−	+	+	+	++
25 mg. ascorbic acid per l.	−	−	−	−	−	−	−
40 mg. ascorbic acid per l.	−	−	−	−	−	?	?

− not oxidized.
+ slightly oxidized.
++ pronounced oxidation.
+++ very pronounced oxidation.
? doubtful.

SUMMARY

In glass containers a 0.004% level of ethyl hydrocaffeate was effective in delaying oxidized flavor development in stored frozen cream for a period of 12 months. In the presence of 0.5 p.p.m. of copper in glass containers, 0.04% and 0.02% concentrations of the antioxidant were effective through a 12-month period, but the 0.004% concentration was effective for a period of only 1 month. In

metal containers the antioxidant was not as effective as in glass, and in the presence of copper only the 0.04% concentration of ethyl hydrocaffeate prevented the oxidized flavor for a 12-month period.

Ascorbic acid at levels of 20, 25, and 40 mg. per liter not only was ineffective in preventing the development but actually accelerated the oxidized flavor. Ascorbic acid added to ethyl hydrocaffeate produced a synergistic effect, so that a 0.004% concentration of ethyl hydrocaffeate and 25 mg. per liter of ascorbic acid prevented the development of the oxidized flavor in cream stored in tinned cans, with and without 0.5 p.p.m. of copper, for a 12-month period.

ACKNOWLEDGMENTS

The authors are indebted to I. I. Rusoff of General Foods Corporation, Hoboken, N. J., for supplying the ethyl hydrocaffeate.

REFERENCES

(1) Bell, R. W. Retention of Ascorbic Acid, Changes in Oxidation-Reduction Potential and the Prevention of an Oxidized Flavor During Freezing Preservation of Milk. *J. Dairy Sci.*, **31**: 951. 1948.

(2) Bell, R. W., and Mucha, T. J. Deferment of an Oxidized Flavor in Frozen Milk by Ascorbic Acid Fortification and by Hydrogen Peroxide Oxidation of the Ascorbic Acid by Fresh Milk. *J. Dairy Sci.*, **32**: 833. 1949.

(3) Brown, W. C., and Olson, F. C. Oxidized Flavor in Milk. XII. Further Studies of the Ascorbic Acid Mechanisms in the Production of Oxidized Flavor in Milk. *J. Dairy Sci.*, **25**: 1041. 1942.

(4) Chieson, W. H., Martin, W. H., and Parrish, D. B. The Relationship of Ascorbic Acid to the Development of Oxidized Flavor in Market Milk. *J. Dairy Sci.*, **32**: 306. 1949.

(5) Gelpi, A. J., Rusoff, L. L., and Skole, R. D. Effect of Antioxidants in the Control of Oxidized Flavor Development in Stored Frozen Cream. I. Use of Ethyl Caffeate, Sustane, Tenox II, and Tenox BHA. *J. Dairy Sci.*, **35**: 93. 1952.

(6) Stull, J. W., Herreid, E. O., and Tracy, P. H. A Study of the Effect of Nordihydroguaiaretic Acid on the Oxygen Absorption of the Phospholipid Fraction of Milk. II. The Effect of Synergists, Metallic Catalysts and pH. *J. Dairy Sci.*, **34**: 187. 1951.

THE EFFECT OF SEMEN DILUTION WITH A YOLK-CONTAINING EXTENDER ON THE OXYGEN UPTAKE AND MOTILITY OF BULL SPERMATOZOA

MARCUS W. H. BISHOP[1] AND G. W. SALISBURY
Department of Dairy Science, Univerity of Illinois, Urbana

Large scale artificial breeding of dairy cattle depends on extending semen with diluents. Survival of mammalian spermatozoa in vitro is adversely affected by dilution, and much attention has been given to the development of extenders that will prolong the functional life-span of the sperm cell.

Many early difficulties were overcome by the introduction of egg-yolk diluents *(6, 7, 8, 13)*, and normal fertility can now be maintained in routine insemination practice when the concentration of spermatozoa is reduced to between 10 and 5 millions of cells per milliliter *(3, 4, 9, 10, 11, 12, 17, 18, 19)*. Such final concentrations may involve extension of 1 part of semen with 200 or more parts of diluent. Even with egg-yolk media the ability of the spermatozoa to live and remain active during storage declines with each increase in dilution *(10, 18, 19)*. Thus it appears that by present techniques the maximal dilution ratios consistent with high fertility have been reached and that further advances must await a more fundamental understanding of the effects of dilution *(11)*.

Previous studies on this problem in mammalian semen have been mainly concerned with assessment of the motility and viability of spermatozoa in different diluents, and few attempts have been made to evaluate it in terms of metabolic activity. Early studies of the "dilution effect" on livability under the essentially anaerobic conditions of artificial breeding practice and 5° C. storage temperature were with the yolk-citrate diluent *(10)*.

Tosic and Walton *(16)* have used yolk-phosphate as a diluent for respiration studies with bull spermatozoa and have found that hydrogen peroxide is formed in amounts toxic to spermatozoa by the oxidative deamination of L-tryptophan, L-tyrosine and L-phenylalanine found in the yolk.

More recently when bull semen was suspended in simple mineral diluents under aerobic conditions at or near body temperature, it was found that a saline-phosphate diluent depressed both respiration and motility of the sperm cells *(2)*. This depression was shown to be due to the phosphate ion.

The experiments reported here were conducted to determine if egg yolk would prevent the depressive effect of phosphate on oxygen uptake and motility of sperm cells and if the dilution effect in the presence of yolk was due to the accumulation of hydrogen peroxide.

Received for publication September 28, 1954.

[1] United Kingdom Fulbright Scholar on leave of absence from the Agricultural Research Council, Unit of Animal Reproduction, School of Agriculture, Cambridge, England.

MATERIALS AND METHODS

The saline-phosphate consisted of equal volumes of saline (0.8 g. NaCl, 0.04 g. $MgCl_2 \cdot 6H_2O$, 0.04 g. KCl, and glass-distilled water to 100 ml.) and *M*/7 phosphate buffer (a mixture of 3.48% $Na_2HPO_4 \cdot 7H_2O$ and 2.35% $NaH_2PO_4 \cdot H_2O$ in proportion to give a pH of 7.0). It was autoclaved before use and designed to be isotonic with bull semen after the recommendations of Foote *(5)*. Equal volumes of this solution and fresh hen egg yolk were mixed for the final diluent.

Catalase, prepared in a crystalline form from beef liver by the method of Sumner and Dounce *(14)*, was added to one half of each batch of the yolk-diluent to prevent the accumulation of hydrogen peroxide. The stock concentrated solution containing ca. 20γ of crystalline catalase per milliliter was stored in the freezing compartment of a refrigerator, diluted 100 times with distilled water immediately before use, and added to the yolk-saline-phosphate in the ratio of 1:10. The untreated diluent received an equivalent amount of distilled water.

The methods used for collection and dilution of semen, and for measurements of oxygen uptake, sperm cell concentration, motility, and pH have been described in an earlier paper *(1)*. In each experiment the same volume of semen (0.5 ml.) from a single ejaculate was placed in each manometer flask so that the oxygen uptake measured in each treatment was for the same number of spermatozoa regardless of the ratio of dilution employed. Samples were diluted in the manometer flasks, by tipping from side arms, either immediately after manometric equilibration or after measurement of oxygen uptake for 1 hour on the undiluted semen. Control measurements of oxygen uptake were made on each diluent, and appropriate corrections have been made for this activity.

RESULTS

Dilution with egg-yolk-saline-phosphate stimulated oxygen uptake (Figure 1) but depressed motility. The rate of oxygen uptake of diluted samples was greatest immediately following dilution and thereafter declined, the decline being greatest in samples without catalase. With catalase the rate of oxygen uptake was usually maintained above that of the undiluted control, but in some cases without catalase it fell well below that of the control (Figure 2). The size of the dilution response varied considerably between ejaculates, as may be seen by comparing the 1:4 dilution treatments without catalase in Figures 1 and 2. In some experiments the oxygen uptake of the egg-yolk diluent was considerable after 2 or 3 hours and increased with time in an exponential manner; appropriate corrections for this activity were made from control observations on the diluent alone.

In four ejaculates (Table 1), which were diluted with egg-yolk-saline-phosphate after 1 hour in the manometer flasks, the mean initial rate of oxygen uptake and the total mean oxygen uptake over 5 hours were increased by dilution, the increase being greater with catalase than without it. The 1:3 dilution treatment with catalase increased oxygen uptake during the first hour following dilution by a mean of 75% and during 4 hours by a mean of 41%. Without

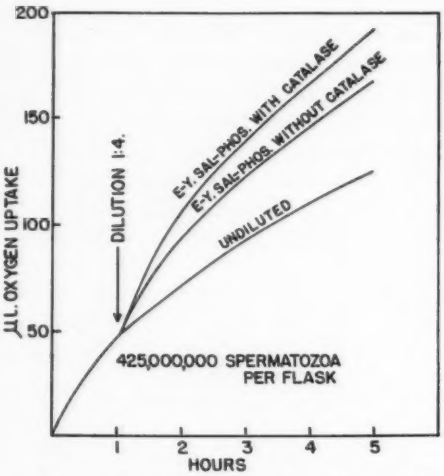


FIG. 1. The effects of a 1:4 dilution with egg-yolk-saline-phosphate diluent (with and without catalase) on the oxygen uptake of 0.5 ml. bull semen.

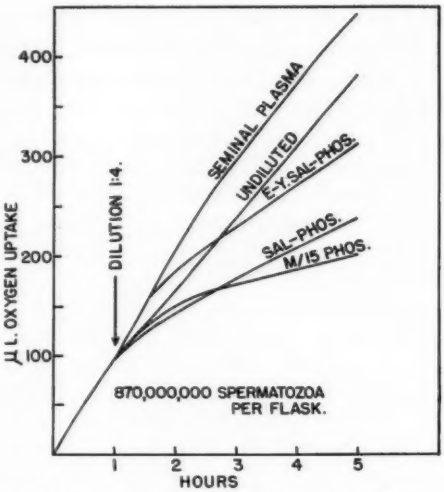


FIG. 2. The effects of a 1:4 dilution with seminal plasma, egg-yolk-saline-phosphate (without catalase), isotonic saline-phosphate and *M*/15 phosphate buffer on the oxygen uptake of 0.5 ml. bull semen.

catalase the mean increases of oxygen uptake for this dilution ratio after 1 hour and 4 hours were 39% and 6%, respectively. The increased rate of oxygen uptake consequent upon dilution was not clearly related to the degree of dilution in samples either with or without catalase. Sperm motility at the end of the experimental period was considerably depressed in the diluted treatments and was not noticeably improved by the presence of catalase. The final motility of the undiluted controls (after dilution with 0.9% sodium chloride) was almost as good after 5 hours in the manometers as at the beginning of the experiments.

TABLE 1

Effect of dilution after 1 hour with egg-yolk-saline-phosphate diluent on oxygen uptake of spermatozoa[a]

Diluent	Dilution ratio	Total O_2 uptake $\mu l/10^9$ spermatozoa					Final motility	Final pH
		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5		
None	1:0	107	184	251	320	380	139	6.53
Without catalase	1:½	115	230	303	373	435	37	6.40
	1:1	—	—	—	—	—	—	—
	1:3	117	224	293	349	406	24	6.58
	1:5	108	219	295	350	408	27	6.65
With catalase	1:½	112	240	339	427	506	62	6.47
	1:1	110	240	321	406	480	28	6.51
	1:3	113	248	329	419	497	30	6.60
	1:5	—	—	—	—	—	—	—

[a] Mean values of observations on 4 ejaculates having a mean sperm concentration of 943 million cells per milliliter and a mean original motility rating of 173 (% motility × rate).

In all cases motility was considerably improved by the addition of 0.9% sodium chloride during the motility examination. The final pH levels did not vary greatly between treatments but were directly related to the degree of dilution.

Similar results were obtained over a greater range of dilution ratios in 50-ml. Warburg flasks with five ejaculates which were diluted immediately after manometric equilibration (Table 2). The mean initial rate of oxygen uptake was greatest in samples without catalase, and during the first hour the oxygen uptake at a dilution ratio of 1:29 was double that of the undiluted controls. After the first hour, however, this rate declined, and after 5 hours the mean total oxygen

TABLE 2

Effect of dilution with egg-yolk-saline-phosphate diluent on oxygen uptake of spermatozoa[a]

Diluent	Dilution ratio	Total O_2 uptake $\mu l/10^8$ spermatozoa					Final motility	Final pH
		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5		
None	1:0	93	184	235	285	366	197	6.44
Without catalase	1:1	157	241	330	378	472	22	6.36
	1:9	146	216	288	342	413	80	6.61
	1:19	163	241	316	376	441	84	6.64
	1:29	181	284	359	426	509	69	6.66
With catalase	1:1	150	258	355	409	500	16	6.39
	1:9	132	253	375	440	546	23	6.63
	1:19	142	275	390	488	595	26	6.66
	1:29	160	296	411	512	618	17	6.66

[a] Mean values of observations on 5 ejaculates having a mean sperm concentration of 998 million cells per milliliter and a mean original motility rating of 197 (% motility × rate).

uptake of the 1:29 treatments without catalase exceeded that of the undiluted controls by only 39% as against a 69% increase for the corresponding treatments with catalase. With the addition of catalase the stimulation of oxygen uptake was directly related to, though not proportionate to, the degree of dilution, but without catalase the 1:1 dilution treatments displayed higher rates of oxygen utilization than did the 1:9 and 1:19 treatments. Despite their increased oxygen uptake, the motility of diluted spermatozoa at the end of the experiments was found to be greatly inferior to that of the undiluted controls, and appeared to be better without catalase than with it and poorest in the 1:1 dilution treatments.

DISCUSSION

In other experiments *(2)* it was shown that saline-phosphate alone as a semen diluent depressed both oxygen consumption and motility of bull spermatozoa under aerobic conditions at 37° C. as compared to that in undiluted semen. The depressive effect was directly related to the concentration of phosphate ion. The results reported here indicate that additions of egg yolk to the saline-phosphate under the same conditions have a mitigating influence on the depressive effect of the phosphate on oxygen uptake but not on that of motility. The beneficial influence on respiration may be solely a consequence of dilution of the phosphate ion by the yolk. Tosic and Walton *(15)* observed that the oxygen-uptake of bull

semen was greater in egg yolk-phosphate than in phosphate alone and that this difference was directly related to the proportion of egg yolk (and therefore inversely related to the proportion of phosphate) in the diluent.

The failure of the increased respiration in the presence of yolk to be reflected in improved motility provides further evidence, earlier found for dilution with seminal plasma *(1)*, that under these circumstances oxygen uptake and motility are not necessarily directly related. However, the apparent inverse relationship of respiration in seminal plasma and in yolk to motility is not due to the respiration resulting in hydrogen peroxide formation in toxic amounts depressing sperm motility for, though catalase improved respiration, no significant beneficial influence on motility was observed when it was added in these experiments. With or without catalase the diluent depressed motility markedly as compared to that in undiluted semen.

The higher levels of oxygen uptake that resulted from the addition of catalase to the egg-yolk diluent support the conclusion of Tosic and Walton *(16)* that in its absence hydrogen peroxide accumulates and depresses respiration. However, a direct relation to motility is not apparent in our work.

SUMMARY

Dilution of semen with egg-yolk-saline-phosphate extender stimulated oxygen uptake by bovine spermatozoa at 37° C. over that observed in undiluted semen, the stimulation being more sustained with the addition of catalase. Motility of the spermatozoa was depressed by the dilution and was not improved significantly by the addition of catalase. Under these conditions oxygen uptake and motility were essentially independent.

ACKNOWLEDGMENT

The authors are indebted to W. A. Wood for preparing the catalase used, to F. N. Baker for collecting most of the semen samples used, to Frank Seidl for help in the laboratory, and to N. L. VanDemark for his aid and counsel.

REFERENCES

(1) Bishop, M. W. H., and Salisbury, G. W. The Effect of Sperm Concentration on the Oxygen Uptake of Bull Semen. *Am. J. Physiol.* (In press).

(2) Bishop, M. W. H., and Salisbury, G. W. The Effect of Dilution with Saline and Phosphate Solutions on the Oxygen Uptake of Bull Semen. *Am. J. Physiol.* (In press).

(3) Branton, C., Kellgren, H. C., and Patrick, T. E. The Importance of Numbers of Spermatozoa in Relation to Semen Quality and Fertility of Dairy Bulls. *J. Dairy Sci.*, **36**: 1301. 1953.

(4) Bratton, R. W. Personal Communication.

(5) Foote, R. H. The Effect of Sulfonamides and Other Bacterial Antagonists upon the Livability of Spermatozoa and upon the Control of Bacteria in Diluted Bull Semen. (Ph.D. Thesis) Cornell University. 1947.

(6) Lardy, H. A., and Phillips, P. H. Preservation of Spermatozoa. *Proc.. Am. Soc. Animal Production*, 32: 219. 1939.

(7) Phillips, P. H. Preservation of Bull Semen. *J. Biol. Chem.*, 130: 415. 1939.

(8) PHILLIPS, P. H., AND LARDY, H. A. A Yolk–Buffer Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, **23**: 399. 1940.

(9) SALISBURY, G. W. Fertility of Bull Semen Diluted at 1:100. *J. Dairy Sci.*, **29**: 695. 1946.

(10) SALISBURY, G. W., BECK, G. H., CUPPS, P. T., AND ELLIOTT, I. The Effect of Dilution Rate on the Livability and the Fertility of Bull Spermatozoa Used for Artificial Insemination. *J. Dairy Sci.*, **26**: 1057. 1943.

(11) SALISBURY, G. W., AND BRATTON, R. W. Fertility Level of Bull Semen Diluted at 1:400 with and without Sulfonilamide. *J. Dairy Sci.*, **31**: 817. 1948.

(12) SALISBURY, G. W., ELLIOTT, I., AND VANDEMARK, N. L. Further Studies of the Effect of Dilution Rate on the Fertility of Bull Semen Used for Artificial Insemination. *J. Dairy Sci.*, **28**: 233. 1945.

(13) SALISBURY, G. W., FULLER, H. K., AND WILLETT, E. L. Preservation of Bovine Spermatozoa in Yolk–Citrate Diluent and Field Results from Its Use. *J. Dairy Sci.*, **24**: 905. 1941.

(14) SUMNER, J. B., AND SOMERS, G. F. *Chemistry and Methods of Enzymes.* 2nd ed., p. 211. The Academic Press, Inc., New York. 1947.

(15) TOSIC, J., AND WALTON, A. Effect of Egg-Yolk and Its Constituents on the Respiration and Fertilizing Capacity of Spermatozoa. *J. Agr. Sci.*, **37**: 69. 1946.

(16) TOSIC, J., AND WALTON, A. Metabolism of Spermatozoa. The Formation and Elimination of Hydrogen Peroxide by Spermatozoa and Effects on Motility and Survival. *Biochem. J.*, **47**: 199. 1950.

(17) WILLETT, E. L. Fertility and Livability of Bull Semen Diluted at Various Levels to 1:300. *J. Dairy Sci.*, **33**: 43. 1950.

(18) WILLETT, E. L. Decline in Fertility of Bull Semen with Increase in Storage Time as Influenced by Dilution Rate. *J. Dairy Sci.*, **36**: 1182. 1953.

(19) WILLETT, E. L., AND LARSON, G. L. Fertility of Bull Semen as Influenced by Dilution Level, Antibiotics, Spermatozoan Numbers and the Interaction of These Factors. *J. Dairy Sci.*, **35**: 899. 1952.

STUDIES ON CORN STEEP LIQUOR IN THE NUTRITION OF CERTAIN LACTIC ACID BACTERIA[1,2]

H. E. KENNEDY AND M. L. SPECK
Department of Animal Industry, North Carolina State College, Raleigh

In the literature on the nutrition of lactic acid bacteria, crude corn steep liquor solids are sometimes mentioned as a nutrient source *(4, 6, 7, 8)*. Corn steep liquor is a by-product of the corn wet-milling industry *(2)*, and to date its use in penicillin production is by far its most important application in the nutrition of microorganisms. Generally speaking, most organisms capable of growing well on simple media containing beef extract and peptone will grow on a medium containing only corn steep liquor *(2)*.

Stiles and Pruess *(6)* showed corn steep liquor to be an accessory nutrient in the lactic acid fermentation of molasses by *Lactobacillus delbrueckii*. Wright *et al. (10)*, in a study of the replacement of the "*L. bulgaricus* factor" (LBF) by orotic acid as a growth factor for *L. bulgaricus*, found corn steep liquor as well as solubilized liver extract to contain the orotic acid activity. Williams *et al. (7)* reported LBF activity in corn steep liquor, but in a much lower concentration than in yeast extract. In studies of vitamin B_{12} in the nutrition of *L. leichmanii* 313, Winsten and Eigen *(8)* observed, by autobiographic studies of paper chromatograms, that corn steep liquor contained at least one alternate growth factor for this organism. By these studies, corn steep liquor was found to contain fractions displaying vitamin B_{12} activity but also was found to lack the vitamin itself.

In view of the above observations, it appeared that corn steep liquor might possess stimulatory factors for the growth of lactic acid-producing bacteria in milk. The present study has shown the presence in corn steep of nutrients for a variety of microorganisms important in milk fermentations. The study was extended to gain more information regarding the types of bacteria affected as well as the effect of corn steep in other media.

EXPERIMENTAL PROCEDURE

Cultures. The cultures used were carried by weekly transfer in litmus milk, and fresh 24-hour cultures were used for inocula in the growth tests. For tests in synthetic media, inocula were prepared by making several transfers in a liquid synthetic medium before making the final transfer for inoculation. *Streptococcus cremoris*, some strains of *S. lactis*, and *Leuconostoc dextranicum* were carried at 20° C. Other strains of *S. lactis* were carried at 32° C. All other

Received for publication September 30, 1954.

[1] Published with the approval of the Director of Research, North Carolina Agricultural Experiment Station, Raleigh, as Paper No. 594 of the Journal Series.

[2] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and N. C. State College (NR 135-111).

cultures were maintained at 35° C. except *S. thermophilus*, which was incubated at 45° C.

Supplementary corn steep liquor preparations. Corn steep liquor is composed of the soluble compounds of corn, which have been extracted by soaking, and is concentrated to about 50% solids content. The concentrated steep liquor used in the starting materials in this study was the grade used by penicillin manufacturers.[3] Four different corn steep liquor preparations were used. Definitions of these preparations are: *(a)* whole corn steep—a representative aliquot of corn steep which was neutralized and diluted to 50% concentration by weight with water; *(b)* 50% supernatant corn steep liquor—corn steep liquor was treated as in *a* but the precipitate was centrifuged off and the clear supernatant liquid decanted for use; *(c)* dialyzate—preparation which was obtained by dialysis of unneutralized 50% corn steep liquor with a dialyzing cellophane membrane against distilled water; dialysis was at room temperature for 24 hours with 3 changes of water; the dialyzate was concentrated to the volume of the original corn steep; *(d)* barium filtrate preparation—a preparation made by adding approximately 165 ml. of 10% $Ba(OH)_2$ to 100 g. of corn steep liquor and warming to 50° C.; the mixture was then filtered and $Ba(OH)_2$ again added until no additional precipitate was formed; after filtration, the excess barium was removed by adding H_2SO_4; the solution was again filtered; the filtrate was treated with charcoal and filtered again.

In the first phase of the study 1% whole corn steep was added to 600 ml. of fresh skimmilk contained in 750-ml. Erlenmeyer flasks. The fortified skimmilk as well as plain skimmilk (control) was then sterilized by autoclaving at 120° C. for 20 minutes. The volume of corn steep liquor was replaced in the controls by sterile distilled water. After the tempering of the flask contents to the temperature at which the test organism was to be grown, 1% of an active (18-24 hours) skimmilk culture was added to the control and to the fortified skimmilk flasks. Aliquots were withdrawn from each flask after 0, 2, 5, 8, 11, and 24 hours incubation for plate counts and titratable acidity determinations (measured as lactic acid) with 0.1 *N* NaOH. The streptococci were plated with Trypticase soy agar (formula by BBL); the leuconostocs and lactobacilli were plated with Trypticase sugar agar (BBL); *Propionibacterium shermanii* and *Pseudomonas aeruginosa* were plated on TGEM agar (Difco) and *Bacillus subtilis* and *Escherichia coli* were plated with nutrient agar (Difco).

Corn steep preparations were tested for stimulation of *L. casei*, *L. bulgaricus*, and *S. lactis* in synthetic or semi-synthetic media. Inocula for tests in synthetic media were prepared by centrifuging cells from a 24-hour culture in synthetic media. The cells were resuspended in an equal volume of saline, and one drop of this suspension was added to each tube for inoculation. Stimulation by corn steep liquor supplements was estimated titrimetrically as well as turbidimetrically.

In order to measure the effects of concentration of corn steep in milk and synthetic media on the growth of *S. lactis* and *L. casei*, 0, 0.5, 1.0, and 1.5% of

[3] Obtained from the Staley Manufacturing Company and Corn Products Refining Company.

whole neutralized corn steep were added. Twenty-five ml. quantities of fresh raw skimmilk were prepared in separate 50-ml. vials. One set was prepared for each incubation period to be tested, and the vials were then autoclaved. Each vial was inoculated with 1% by volume of an 18-24 hour skimmilk culture of the test organism. Synthetic media were prepared and inoculated as described above. *S. lactis* 27 was incubated at 32° C. and *L. casei* 7469 at 35° C. Growth in milk was measured by titratable acidity (as lactic acid) after 6, 8, 10, 13, and 22 hours. One set of vials was titrated at the beginning of the experiment to account for the titratable acidity contributed by the milk and corn steep. Measurements of growth in synthetic media were made turbidimetrically with a 580-mμ filter and a Lumetron colorimeter.

RESULTS AND DISCUSSION

Stimulation of bacteria in milk. Undesirable organisms which may be present in milk were studied, as well as desirable lactic acid bacteria, since both types may be stimulated by the addition of growth stimulants to milk. Representatives of acid and flavor-producing groups of bacteria used in starter cultures were found to be stimulated by the addition of 1% corn steep liquor to the milk medium. The effect of corn steep on growth and titratable acidity produced by the organisms in milk is shown in Table 1. The growth of both *S. lactis* 27 and *S. cremoris* ML1 was stimulated in milk by corn steep liquor. *S. lactis* 27, which is a rapid acid-producer in milk, showed very significant response to corn steep liquor by an increased rate of growth and acid production and higher total acid and total populations. The rate of acid production by *S. cremoris* ML1 was stimulated by the addition of 1% corn steep to milk. However, no appreciable difference was observed as measured by colony counts. The apparent discrepancy

TABLE 1

The effect of corn steep liquor on bacteria in milk

Organism	Hours incubation	Per cent titratable acidity		Log of plate count	
		Control	Corn steep	Control	Corn steep
S. lactis (27)	0	0.20	0.25	6.95	7.00
	2	0.20	0.27	7.00	7.03
	5	0.25	0.39	8.52	8.76
	8	0.30	0.80	8.92	9.34
	11	0.46	0.95	9.06	9.33
	24	0.74	1.08	8.91	9.15
S. cremoris (ML1)	0	0.18	0.22	7.04	7.06
	2	0.18	0.24	7.56	7.57
	5	0.24	0.44	8.48	8.65
	8	0.34	0.77	9.02	8.91
	11	0.65	0.86	9.10	9.06
	24	0.88	0.97	9.16	8.91
S. thermophilus (7)	0	0.20	0.24	6.32	6.44
	2	0.20	0.25	6.71	6.71
	5	0.39	0.63	7.39	7.65
	8	0.52	0.80	8.79	9.16
	11	0.63	0.94	8.78	9.01
	24	0.83	1.00	7.85	7.75

TABLE 1 (concluded)

The effect of corn steep liquor on bacteria in milk

Organism	Hours incubation	Per cent titratable acidity		Log of plate count	
		Control	Corn steep	Control	Corn steep
L. casei (7469)	0	0.20	0.22	6.73	6.72
	2	0.20	0.24	6.95	6.99
	5	0.24	0.28	7.54	7.60
	8	0.24	0.34	7.61	8.40
	11	0.24	0.50	7.80	8.58
	24	0.42	1.24	8.93	9.51
L. bulgaricus (LB2)	0	0.22	0.26	7.22	7.18
	2	0.24	0.30	7.14	7.21
	5	0.28	0.46	7.40	7.84
	8	0.34	1.06	8.57	8.88
	11	0.50	1.48	8.92	9.17
	24	1.24	2.44	8.97	9.10
L. arabinosus (8014)	0	0.18	0.22	5.40	5.48
	2	0.18	0.22	5.81	6.00
	5	0.16	0.22	6.74	7.37
	8	0.14	0.24	6.88	8.45
	11	0.18	0.28	7.41	8.76
	24	0.26	0.98	7.81	9.26
Prop. shermanii (8262)	0	0.20	0.23	5.80	5.70
	2	0.20	0.24	5.69	5.74
	5	0.20	0.24	6.69	6.81
	8	0.20	0.24	7.15	7.36
	11	0.20	0.28	7.38	8.30
	24	0.21	0.34	7.75	8.17
Leuc. citrovorum (8081)	0	0.20	0.24	5.27	5.38
	2	0.20	0.25	5.30	5.38
	5	0.20	0.25	5.44	5.53
	8	0.20	0.25	5.78	5.87
	11	0.20	0.25	6.43	7.00
	24	0.20	0.25	7.26	8.13
Leuc. dextranicum (8358)	0	0.21	0.26	6.13	6.11
	2	0.21	0.26	6.58	6.54
	5	0.23	0.27	6.89	7.24
	8	0.21	0.28	7.36	7.64
	11	0.21	0.29	7.78	8.26
	24	0.21	0.39	8.20	8.64
B. subtilis (102 Ford)	0			5.44	5.25
	2			5.62	5.44
	5			6.24	5.81
	8			7.15	6.35
	11			7.24	7.05
	24			7.49	7.61
Ps. aeruginosa (142)	0			6.98	7.03
	2			7.29	7.23
	5			7.90	7.99
	8			8.24	8.49
	11			8.71	8.49
	24			9.23	9.26
E. coli (NCDH)	0	0.21	0.25	6.76	6.86
	2	0.21	0.26	6.97	7.04
	5	0.25	0.35	7.96	8.30
	8	0.35	0.52	8.53	8.86
	11	0.44	0.62	8.70	8.96
	24	0.63	0.66	8.58	8.60

may be explained by preliminary observations, which have indicated that corn steep effects much longer chain formation in cultures grown in the presence of the corn steep supplement. These observations have also indicated that larger cells are formed in such supplemented cultures.

The response of *S. thermophilus* to corn steep liquor, as measured by titratable acidity, was very similar to that of *S. lactis*. The rate of growth was appreciably stimulated, although there was little difference in the total population at the end of the incubation period.

Although *L. bulgaricus* is a rapid acid producer in milk, when corn steep was added the lag phase was shortened, the rate of acid production was greatly increased, and the total acid produced in 24 hours was about doubled. In the stimulated sample the period of most rapid acid production was between the fifth and eighth hours, whereas in the control the period of most rapid acid production was after the eighth hour of incubation.

As with the other lactobacilli, *L. casei* produces high acid in milk, but at a slower rate. The culture studied usually produces 0.4 to 0.5% titratable acidity in milk in 24 hours. When milk was supplemented with 1% corn steep, a titratable acidity of 1.24% was obtained in 24 hours. Also, the rate of growth and total bacterial population were greatly increased.

Corn steep liquor had greater effect on the increase in populations for *L. arabinosus* than for any other organism studied in this series. The rate of acid production also was greatly increased.

P. shermanii, *L. citrovorum*, and *L. dextranicum* responded significantly to corn steep liquor, as measured by plate counts. Little difference in acid production was observed, as might be expected since these organisms are not high acid producers. It does seem reasonable, however, to expect a greater amount of propionic acid to be produced by the *P. shermanii* culture and more diacetyl to be produced by the leuconostocs, since there are greater numbers of these organisms in cultures supplemented with corn steep liquor.

E. coli showed some increase in rate of growth and rate of acid production by the corn steep liquor, but at the end of 24 hours, the total bacterial count was approximately the same, and the total titratable acidity was almost the same, as that of the control. Since *E. coli* is considered a pseudo-lactic acid organism, it is not surprising that it was stimulated to some extent by corn steep liquor. The rate of growth of *B. subtilis* actually seemed to be reduced by the presence of corn steep, and no effect at all was shown on the growth of *P. aeruginosa*. Indications are that corn steep liquor contains a nutrient specific for acid-producing and certain flavor-producing bacteria, and that it is not of nutritional importance to common proteolytic types of bacteria in dairy products.

It is especially interesting from the standpoint of possible applications in the dairy industry that, of the bacteria tested, only members of the family *Lactobacteriaceae* showed marked stimulation by corn steep liquor. Thus, corn steep, treated to rid it of its objectionable flavor or purification of the stimulatory factor(s) contained in it, would appear to offer promise in accelerating fermenta-

tions involved in the manufacture of cultured buttermilk, cottage cheese, and various other cheeses.

Stimulation of bacteria in synthetic or semi-synthetic media. Experiments were designed to determine whether corn steep stimulated the growth of *L. casei* in synthetic or semi-synthetic media that are reportedly complete for this organism. The medium of Rickes *et al. (4)* and that of Roberts and Snell *(5)*, both of which are considered to contain all of the essential and accessory growth factors for *L. casei*, were studied. Representative data of repeated experiments are shown in Table 2. Consistent results were obtained when corn steep was added

TABLE 2

Stimulation of L. casei *by corn steep liquor in synthetic media*

Per cent corn steep	Media	
	Roberts and Snell	Rickes *et al.*
	(Per cent transmission)[a]	*(Per cent transmission)*[b]
0.0	72	60
0.05	59	49
0.10	52	44
0.50	45	36
1.00	41	30

[a] 10% supernatant corn steep liquor, cultures incubated 12 hours.
[b] 50% corn steep dialyzate, cultures incubated 11 hours.

to the medium of Roberts and Snell. The order of growth in the medium of Rickes *et al.* was less consistent than in Roberts and Snell's medium, but in every instance very marked stimulation was shown by the addition of corn steep liquor.

Presumably the rate of synthesis of strepogenin is the chief factor limiting the rate of growth of *L. casei* in a medium containing acid hydrolyzate of casein or pure amino acids as the source of nitrogen. Rickes *et al.* concluded that the increase in growth rate observed when serine, asparagine, and glutamic acid were supplied to the test organism was due to an increased rate of strepogenin synthesis. Glutamic acid and possibly serine have been postulated as components of the strepogenin molecule *(9)* and thus are presumably precursors in the synthesis of strepogenin. A medium of this type, completely defined chemically, was especially desirable in the present work for testing for unknown stimulatory substances; it was found to be unsatisfactory, however, because consistent growth could not be obtained when it was used. The medium of Roberts and Snell is synthetic except for the enzymatic hydrolyzate of casein, which is a source of strepogenin. Results in this medium were consistent, and the stimulatory effect of corn steep therein was repeatedly demonstrated. The effectiveness of corn steep liquor in further increasing the rate of growth of *L. casei* in this medium is presumably due to a factor(s) other than those recognized as essential for rapid and optimum growth.

Williams *et al. (7)* reported the presence of LBF activity in corn steep liquor when tested in their medium. In the present study the growth of *L. bulgaricus* LB2 was stimulated, however, by corn steep liquor added to the medium of

Williams *et al.* fortified with LBF. This observation indicates the presence of a factor different from LBF, and further evidence of a new stimulatory factor for this species is shown.

Eight strains of *S. lactis* were tested for stimulation by the addition of corn steep liquor to the medium of Niven *(3)* as modified by Collins *et al. (1)*. The corn steep preparation was added aseptically to the medium, and growth was estimated turbidimetrically with a Klett-Summerson colorimeter. Three of the tested strains grew very rapidly. Turbidimetric readings were made on these cultures after 7 hours incubation. The slower growing cultures were read after 17 hours incubation. Every strain of *S. lactis* studied was stimulated by the

TABLE 3

Stimulation of S. lactis *cultures by corn steep liquor in modified Niven's medium*

S. lactis strain number	Klett-Summerson reading		
	No corn steep	0.1% corn steep	Amount of increase
	7 hours incubation		
122.1	17	42	27
24	26	51	25
27	74	99	25
	17 hours incubation		
H	98	162	64
H-1-10	152	178	64
5	126	171	45
144F	108	174	66
ML1	160	218	58

addition of 1.0% corn steep dialyzate to the semi-synthetic medium (Table 3). This stimulation indicates that the medium is not nutritionally complete. Since each culture grew in the unsupplemented medium, stimulation by corn steep liquor is probably not due to an essential metabolite but to a stimulatory factor.

The response of *L. casei* and *S. lactis* to increments of corn steep in milk and synthetic media is shown in Figure 1. *L. casei* showed a positive response to increasing amounts of corn steep when it was added to milk. The response was negligible until after 10 hours incubation, and the most pronounced effect of the stimulant was observed after 22 hours incubation. Relatively greater responses were observed in concentrations between 0 and 0.5% corn steep, although the organism responded to increments up to 2.0%. The range of corn steep activity for *L. casei* in Roberts and Snell's medium was found to be between 0 and 1%. The most pronounced stimulation of growth occurred with concentrations up to 0.25%, after which there was a retardation in response.

S. lactis 27 grew very rapidly in milk. An appreciable response to corn steep was noticed after 6 hours incubation, but the order of response was greater in 10 hours than at any other incubation time (Figure 1). The greatest response of *S. lactis* 27 to increments of corn steep in Niven's medium containing oleate and acetate *(1)* was at concentrations less than 0.1%.

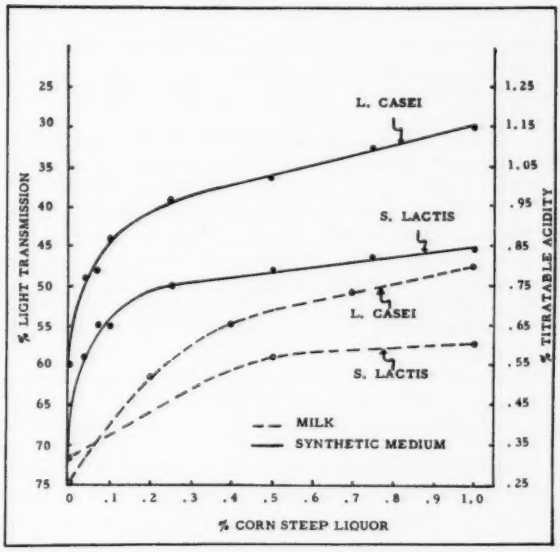


FIG. 1. The response of *L. casei* and *S. lactis* to increments of corn steep liquor in milk and synthetic media.

Because of the regularity in response of *L. casei* and *S. lactis* to corn steep increments, it was possible to develop dose-response curves which may be modified to make a dependable assay technique for the quantitative assay of corn steep activity.

SUMMARY

The addition of 1% corn steep liquor to a milk medium resulted in stimulation of growth of *S. lactis, S. cremoris, S. thermophilus, L. casei, L. arabinosus, L. bulgaricus, L. citrovorum, L. dextranicum,* and *P. shermanii.* Stimulation of *E. coli* was questionable, and *B. subtilis* and *P. aeruginosa* were not stimulated.

L. casei, L. bulgaricus, and *S. lactis* were stimulated by the addition of corn steep preparations to synthetic media which have been reported as complete for the optimum growth of each organism.

A positive relationship was obtained between increments of corn steep added and the amount of response of *L. casei* and *S. lactis* in milk and synthetic media. The greatest response, per unit of corn steep added, was obtained at concentrations below 0.1% in synthetic media.

From the present study, it is indicated that corn steep liquor contains a growth stimulant(s) for certain bacteria used in the production of fermented dairy products. Should the corn steep factor(s) be made available in a purified form, it would be of use in certain dairy plant operations to increase the rate of fermentations and thus shorten fermentation periods.

REFERENCES

(1) COLLINS, E. B., NELSON, F. E., AND PARMELEE, C. E. Acetate and Oleate Requirements of the Lactic Group of Streptococci. *J. Bacteriol.*, **59**: 69. 1950.

(2) LIGGETT, R. W., AND KOFFLER, H. Corn Steep Liquor in Microbiology. *Bacteriol. Revs.*, **12** (4): 297. 1948.

(3) NIVEN, C. R., JR. Nutrition of *Streptococcus lactis*. *J. Bacteriol.*, **47**: 343. 1944.

(4) RICKES, E. L., KOCH, P. J., AND WOOD, T. R. Additional Observations on the Rate of Growth of *Lactobacillus casei*. *J. Biol. Chem.*, **178**: 103. 1949.

(5) ROBERTS, E. C., AND SNELL, E. E. An Improved Medium for Microbiological Assays with *Lactobacillus casei*. *J. Biol. Chem.*, **163**: 499. 1946.

(6) STILES, H. R., AND PRUESS, L. M. Nutrient Requirements of *L. delbruckii* in the Lactic Acid Fermentation of Molasses. *J. Bacteriol.*, **36**: 149. 1938.

(7) WILLIAMS, W. L., HOFF-JORGENSEN, E., AND SNELL, E. E. Determination and Properties of an Unidentified Growth Factor Required by *Lactobacillus bulgaricus*. *J. Biol. Chem.*, **177**: 933. 1949.

(8) WINSTEN, W. A., AND EIGEN, E. Paper Chromatography of Vitamin B_{12} and Related Bacterial Growth Factors. *J. Biol. Chem.*, **181**: 109. 1949.

(9) WOOLLEY, D. W. Strepogenin Activity of Derivatives of Glutamic Acid. *J. Biol. Chem.*, **172**: 71. 1948.

(10) WRIGHT, L. D., HUFF, J. W., SKEGGS, H. R., VALENTIK, K. A., AND BOSSHARDT, D. K. Orotic Acid, a Growth Factor for *Lactobacillus bulgaricus*. *J. Am. Chem. Soc.*, **72**: 2312. 1950.

THE INFLUENCE OF SOME SURFACTANTS UPON THE SELF-DISPERSION AND CHURNING OF WHOLE MILK POWDER[1,2]

D. W. MATHER[3] AND H. A. HOLLENDER
Dairy Department, Purdue University, Lafayette, Indiana

The ease of dispersion of whole milk powder is of paramount importance in its reconstitution for beverage consumption. To date, the dairy industry has failed to manufacture a whole milk powder that can be easily dispersed in water without mechanical agitation. Commercial milk powders are not "wetted" readily, and consequently the powder "balls up," making further dispersion difficult. The production of a high quality, easily dispersible whole milk powder would be of great value to the Armed Forces.

Hibbs and Ashworth *(4)* have reported on the use of emulsifiers and protein stabilizers in whole milk powder. Sorbitan monostearate, polyoxyethylene sorbitan monostearate, glyceryl monostearate, and mixed monoglycerides of cottonseed oil were the surface active compounds or emulsifiers studied. They found that the fat in the powder containing polyoxyethylene sorbitan monostearate had greater tendency to churn when the powder was reconstituted immediately than when the powder had been stored for 1 month at 85° F. The other surface active compounds were found to improve the solubility when stored at 85° F.

Gibson and Raithby *(3)* added the two surface active agents, sorbitan monolaurate and polyoxyethylene sorbitan monolaurate, to condensed skimmilk prior to drying in concentrations of 1% of the weight of nonfat dry milk solids. They found that these surfactants increased the wettability considerably. However, sorbitan monolaurate alone did not appreciably affect the wettability.

This study was undertaken to evaluate some of the surface active compounds as a means of increasing the self-dispersion of whole milk powder.

METHODS

Manufacturing procedure. The milk used for the experimental powders was obtained from the Purdue University Creamery and was of mixed herd origin. The raw milk was standardized to 3.4% butterfat and then preheated to 175° F. for 20 minutes in a stainless steel steam-jacketed kettle. The milk then was condensed to approximately 38% total solids in a Rogers 16-in. stainless steel

Received for publication August 12, 1954.

[1] This paper reports research sponsored by the Quartermaster Food and Container Institute for the Armed Forces and has been assigned No. 493 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

[2] Published with the approval of the Director of the Purdue Agricultural Experiment Station as Journal Series Paper No. 806.

[3] Present address: National Dairy Products Co., Inc., New York.

vacuum pan. The condensed milk was divided into lots, cooled, and held over night at 40° F. The amount of surfactant used was calculated on the fluid milk basis (3.4% butterfat) but added to the concentrated milk prior to homogenization. The surfactants which were not readily dispersible were first emulsified with a small quantity of water by means of a hand-operated laboratory homogenizer. A total concentration of 0.1% (fluid basis) generally was used. One lot, homogenized but without added surfactants, served as a control for that group. The concentrated milk with or without the surfactant was heated to 130° F. and homogenized with a Cherry-Burrell homogenizer at 2,500 p.s.i. The individual lots were cooled in ice water to 50° F. Just prior to drying, each lot was warmed to 100-110° F. The lots were dried on a Swenson research spray drier employing air atomization with an inlet air temperature of approximately 300° F. and an outlet air temperature ranging from 175-210° F. The powder was air-packed in 2-11/16 in. × 3 in. sanitary cans.

Determination of self-dispersion of whole milk powder. The terms "self-dispersion" and "solubility" are similar but different dispersing properties of milk powder. Self-dispersion is related to the rate or ease of dispersion of milk powder without aid of external energy. Solubility is commonly referred to as the relative amount of solids which are dispersed when powder is mechanically

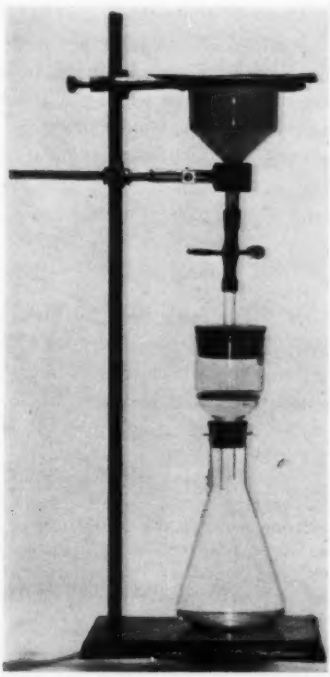


FIG. 1. Apparatus for the determination of the rate of self-dispersion.

mixed with water. For example, the term "solubility index" as used by the American Dry Milk Institute in the grading of dried milk *(1)* is indicative of the amount of undispersed solids which remains after a sample has been reconstituted under certain specified conditions, one of which is the use of high-speed agitation.

The rate of self-dispersion was determined by the method of Stone and Wood *(5)* as modified slightly in this laboratory. The apparatus used is shown in Figure 1.

Ninety ml. of distilled water (75° F.) was contained in the porcelain Buchner funnel by closing the rubber tubing between the funnels with a pinch clamp. Fifteen to 40 g. of powder previously tempered at 86° F. for 12 hours was sifted through the screen as quickly and evenly as possible and allowed to stand for 3 minutes. (Note: The sample size was adjusted so that an excess of powder always was present on the water's surface after the 3-minute contact time.) After the contact period, the clamp was released and the redispersed portion was filtered through the two funnels into the suction flask by vacuum. The filtrate then was poured into a 200-ml. volumetric flask and made up to volume with distilled water. A 10-ml. aliquot was pipetted from the volumetric flask, placed in a tared 50-ml. beaker, dried at 75° C. for 24 hours, and weighed. The weight of powder self-dispersed was calculated on the basis of the total solids in the aliquot, and the data were reported as milligrams per square centimeter of water surface area. Duplicate determinations were made on each lot of powder. Ranges in self-dispersion that were considered to be in the categories of low, medium, and high are listed below:

	mg/cm²	*grams recovered*
Low	7.5	0.396
Medium	58.6	3.076
High	113.6	5.974

Determination of rate of churning. The American Dry Milk Institute solubility index mixing procedure *(1)* was used for determining the rate of churning. The disappearance of the foam during the mixing period served to indicate when the churning was about to take place. The time was noted when the churned butterfat was observed and recorded to the nearest 15 seconds.

RESULTS AND DISCUSSION

A. *Effect of varying concentrations of surfactant upon self-dispersion and churning of the fat during reconstitution of the powder.* The first lots of powder, each with a different surfactant added, exhibited a tendency to churn when reconstituted. The clumps of churned butterfat rose to the surface, making the reconstituted milk unfit for beverage purposes because of its appearance. Because this defect was encountered with the surfactants that were expected to produce the most beneficial results, a large portion of the work was devoted to controlling the defect and attempting to ascertain its mode of action.

The surfactant polyoxyethylene sorbitan monooleate[4] at a concentration of

[4] Atlas Powder Co. Tween 81—from here on referred to as PSMO.

0.1% (fluid basis) was very effective in producing a dispersible powder. However, it possessed the undesirable characteristics of causing churning when the powder was reconstituted with mechanical agitation. To determine the level of concentration at which churning would be evident, a series of powders was prepared with varying concentrations of PSMO.

As shown in Table 1, the first evidence of churning was noted at the 0.10% level. Higher concentrations produced more rapid churning.

TABLE 1

Relation of the amount of polyoxyethylene sorbitan monooleate in whole milk powder to the self-dispersion of the powder and the churning time of the reconstituted milk

Lot No.	Additive	Self-dispersion	Churning time
		(mg/cm²)	
48	Control	61.9	No churning
49	PSMO[a] (0.02%)	118.7	No churning
50	PSMO (0.06%)	123.4	No churning
51	PSMO (0.10%)	147.2	60 seconds
52	PSMO (0.15%)	154.2	45 seconds
53	PSMO (0.20%)	125.7	30 seconds

[a] Polyoxyethylene sorbitan monooleate, Atlas Powder Co., Tween 81.

B. *Effect of varying the concentration and combinations of surfactants as a means of increasing the self-dispersion of the powder and controlling the churning defect.* According to published technical data *(2)* on certain of these surface active compounds, the sorbitan fatty acid esters are classified as "lipophilic" and the polyoxyethylene sorbitan fatty acid esters as "hydrophilic." This technical literature also suggests that certain combinations of compounds may prove more effective than one compound.

Therefore, varying concentrations of a combination of two surfactants were studied as means of controlling the churning. The results indicated (Table 2) that a low concentration of sorbitan monostearate[5] (0.02%) and a high concentration of polyoxyethylene sorbitan monostearate (0.08%)[6] produced the most dispersible powder, but churning occurred. Lot 71, which contained SMS (0.06%) and PSMS (0.04%), was the most dispersible powder that did not churn when reconstituted. Although the data in Table 1 indicate that 0.06% of polyoxyethylene sorbitan monooleate produced a more dispersible powder than did the combination of sorbitan monostearate and polyoxyethylene sorbitan monostearate (Lot 71), the poorer keeping quality of the oleic acid ester ruled out the practical use of this compound. Lot 73 (Table 2) contained 0.10% SMS and 0.10% PSMS, which was double the total concentration of surfactant employed in most previous lots of powder. This increased concentration of surfactant failed to produce a proportional increase in the rate of self-dispersion of the powder. However, as in Lot 71, the churning tendency was eliminated by the combination of the two surfactants.

[5] Atlas Powder Co. Span 62—from here on referred to as SMS.

[6] Atlas Powder Co. Tween 60—from here on referred to as PSMS.

TABLE 2

The effect of combinations of a lipophilic and hydrophilic surfactant upon the self-dispersion of whole milk powder and the churning time of the reconstituted milk

Lot No.	Additive	Self-dispersion	Churning time
		(mg/cm²)	
68	Control	75.1	No churning
69	SMS[a] (0.02%) PSMS[b] (0.08%)	151.2	60 seconds
70	SMS (0.04%) PSMS (0.06%)	119.8	90 seconds
71	SMS (0.06%) PSMS (0.04%)	106.6	No churning
72	SMS (0.08%) PSMS (0.02%)	60.6	No churning
73	SMS (0.10%) PSMS (0.10%)	101.9	No churning

[a] Sorbitan monostearate, Atlas Powder Co., Span 62.
[b] Polyoxyethylene sorbitan monostearate, Atlas Powder Co., Tween 60.

C. *Effect of various additives upon the self-dispersion and churning time of the reconstituted milk.* Table 3 illustrates that some surfactants increase the self-dispersion properties of whole milk powder, whereas others may reduce the rate slightly. The polyoxyalkylene fatty acid ester[7] did not prove beneficial in Lot 40 but did prove beneficial in other lots. No explanation was found for the rather erratic results occasionally obtained with some of the surfactants.

The two protein stabilizers, sodium citrate and disodium phosphate, failed to enhance the rate of self-dispersion of the powder.

D. *Visual observations on the self-dispersing characteristics of whole milk powder with and without added surfactants.* Figure 2 clearly illustrates the beneficial effect that can be achieved when the proper surface active agent is present in whole milk powder.

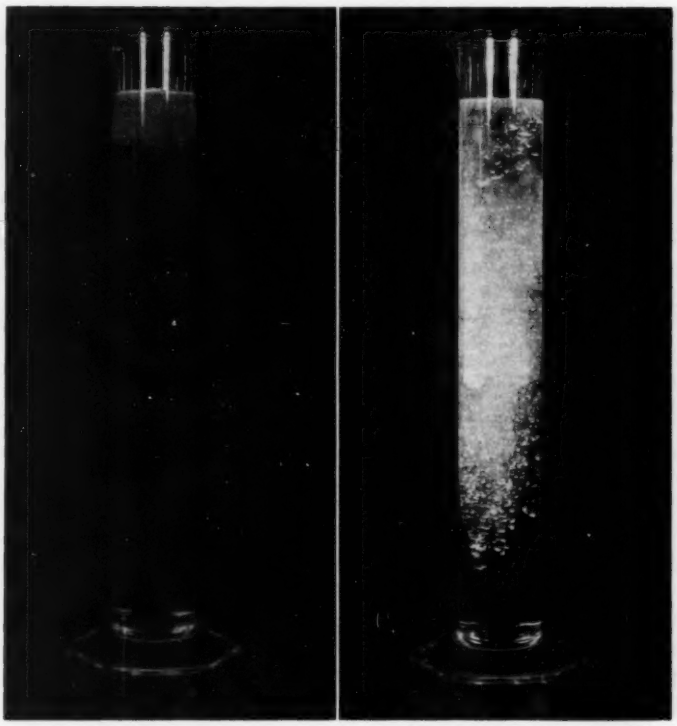
TABLE 3

Relation of various surfactants upon the self-dispersion of whole milk powder and the churning time of the reconstituted milk

Lot No.	Additive	Self-dispersion	Churning time
		(mg/cm²)	
36	Control	42.5	No churning
37	SMS[a] (0.05%) PSMS[b] (0.05%)	87.8	No churning
38	SMS (0.05%) PSMS (0.05%) Sodium citrate (0.10%)	61.3	No churning
39	SMS (0.05%) PSMS (0.05%) Disodium phosphate (0.10%)	32.0	No churning
40	Polyoxyalkylene fatty acid ester[c] (0.10%)	37.2	90 seconds
41	PG 400 MS[d] (0.10%)	30.3	No churning
42	PG 400 MO[e] (0.10%)	43.2	No churning

[a] Sorbitan monostearate, Atlas Powder Co., Span 62.
[b] Polyoxyethylene sorbitan monostearate, Atlas Powder Co., Tween 60.
[c] Alrose Chemical Co., Nonisol 300.
[d] Glyco Products Co., Polyethylene glycol 400 (mono) stearate.
[e] Glyco Products Co., Polyethylene glycol 400 (mono) oleate.

[7] Alrose Chemical Co., Nonisol 300.



Lot 176. Control. Lot 179. Surfactant. (C 1500 MS)

FIG. 2. Dispersing characteristics of whole milk powder as influenced by the addition of surface active agent.

A 1-g. sample of whole milk powder was screened onto the surface of distilled water (75° F.) contained in a glass cylinder (4.8 cm. × 37.7 cm.). The powder temperature was 86° F. Both pictures were taken 17 seconds after the screening process was initiated. The surfactant was carbowax 1500 monostearate[8] added at the rate of 0.10%. The initial rate of self-dispersion, as measured by the method described, was 65.3 and 317.9 mg. per square centimeter for Lots 176 and 179, respectively.

The powder was wetted very quickly once the powder particles touched the water's surface, with subsequent sinking which was accompanied by further dispersion as the particles made their descent. In highly dispersible powders this "wetting" action quickly allowed the water to penetrate the powder particle and the interstices between the powder grains, driving out the air and permitting the powder to sink. The dispersion was of a different nature also, consisting of large clumps of powder as contrasted to the nonsurfactant powder, which displayed minute powder particles. The wetting action which is a prime requisite

[8] Glyco Products Co., Peg. 42—from here on referred to as C 1500 MS.

in the dispersion of whole milk powder was practically nonexistent with the nonsurfactant powder, as exemplified by the layer of undispersed powder on the water's surface.

This surfactant powder also churned to a very slight degree when reconstituted. However, this defect was eliminated by lowering the concentration of C 1500 MS to 0.08% and adding a lipophilic surfactant glyceryl monostearate[9] in a concentration of 0.02%. This did not alter the dispersing characteristics of the powder to any appreciable extent.

SUMMARY

The addition of certain surface active compounds to concentrated milk before spray drying increased the rate of self-dispersion of the milk powder. Surface active compounds which are polyoxyethylene fatty acid esters have been found the most effective but cause churning at certain concentrations.

Sorbitan fatty acid esters were found to be effective in controlling the churning of the butterfat when the powder was reconstituted. When the proper hydrophilic-lipophilic balance of surfactants was present in the whole milk powder, a powder was made that was self-dispersible and yet did not churn when reconstituted.

[9] Glyco Products Co., Aldo 33.

REFERENCES

(1) American Dry Milk Institute, Inc. The Grading of Dry Whole Milk and Sanitary and Quality Standards Including Methods of Analysis. Am. Dry Milk Inst., *Bull.* 913. 1947.

(2) Atlas Powder Company. *Atlas Surface Active Agents.* Atlas Powder Co., Wilmington, Del. 1950.

(3) Gibson, D. L., and Raithby, J. W. Studies on Improving the Ease of Reconstitution of Skim Milk Powder. *Can. J. Technol.*, **32**: 60. 1954.

(4) Hibbs, R. A., and Ashworth, U. S. The Solubility of Whole Milk Powder as Affected by Protein Stabilizers and Emulsifiers. *J. Dairy Sci.*, **34**: 1084. 1951.

(5) Stone, W. K., and Wood, K. R. Dispersing Characteristics of Whole Milk Powder. *Activities Report on Food and Container Research and Development for the Armed Forces*, **5** (2): 116. Published by Research and Development Associates, Food and Container Institute, Inc., Chicago. 1953.

TECHNICAL NOTES

Milk Utilization in Japan

It might be of interest to show how the quality of cow's milk in Japan has changed since World War II. At the war's end, the country was in great need of food. It was even necessary to use some of the cattle feed for human consumption. For example, soya-beans, sweet potatoes including the leaves and runners, bran, and wild grass were commonly used for human food, resulting in a shortage for animal use. People, as well as animals, suffered from poor nutrition. Animals were slaughtered and decreased in numbers.

However, with the coming of the occupation forces, rice, wheat flour, dried skimmilk, butter, soya-beans, coconut, and soya-bean flour, were imported so that the people could obtain the minimum food needed. As a result, more feed for animals was available, and fewer cattle had to be slaughtered. This, together with the importation of some cattle, raised the total number. Furthermore, those which formerly could not be bred because of nutritional deficiency recovered their health and were able to reproduce.

Because of the shortage that existed at the close of the war, the use of milk and milk products was limited to infants and invalids. But the supply increased gradually and by 1950 dairy products were removed from control.

To date, the number of cows in Japan has increased to 350,000, which is about twice the number there were at the end of the war. The amount of milk production has increased to more than that before the war and consumption has increased. Tokyo metropolis at the close of the war had a daily supply of only 390 kg. of cow's milk, but now it has 240,500 kg. The occupation forces imported and distributed milk products, resulting in a greater liking for milk on the part of the Japanese people. The country is very grateful to the occupation forces for bringing this about.

The results of a study made in 1949 are given in Table 1, and results of a more recent investigation made in the Tohoku region are given in Table 2. These data show that cow's milk in Japan is lower in milk solids-not-fat than the milk of other countries. This is probably due to the deficiency in nutrition of the cows, especially protein.

Most cows in Japan are Holsteins, but lately some Jersey animals have been imported. A study made of the quality of the Jersey milk for a period of 8 months showed it to be lower in solids than that of the milk from the same breed in other countries. It is thought that through better feeding practices the solids content of the milk produced by Japanese cows will increase.

Japan lost about half its territory as the result of war and consequently is greatly in want of food. An attempt is being made to bring under cultivation waste lands which previously have not been utilized and to improve grassland and pasture. It is hoped to increase food production, especially the supply of protein, by increased and improved dairying, and eventually to make the country self-supporting. The continued assistance of the United States in bringing about an improved dairying in Japan will be appreciated by her people.

TABLE 1

Composition of raw milk in Japan (1949)

	Specific gravity	Water	Fat	Protein	Lactose	Ash	S.N.F.
		(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Minimum	1.0273	88.06	2.70	2.40	4.03	0.50	7.25
Maximum	1.0326	89.60	3.52	3.28	4.80	0.76	8.68
Average	1.0300	88.93	3.10	2.88	4.35	0.68	7.94

TABLE 2

Composition of raw milk in Japan (1953)

	Specific gravity	Water	Fat	Protein	Lactose	Ash	S.N.F.
		(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Minimum	1.0292	88.45	2.93	2.61	4.05	0.50	7.39
Maximum	1.0322	89.60	3.70	3.12	4.50	0.71	8.10
Average	1.0307	88.97	3.25	2.88	4.28	0.61	7.77

TAKEO NAKANISHI
Tohoku University, Sendai, Japan

PEOPLE and EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

As we judge the success of the leaders and pioneers of our industry, we often fail to appreciate the factors that contributed to their success, the sacrifices they have made or the depth of their contributions to society. Certainly this is true in the case of Professor Emeritus ANDREW A. BORLAND of The Pennsylvania State University.

A. A. Borland

Professor Borland was born on a modest, but progressive, farm at Sandy Lake, Pa. He attended the public schools, but when the time came for college, he found himself without funds to further his education. In 1898 he became a public school teacher (country one-room school) at $30 a month, a position he held for 7 years. At 27 years of age and with a few dollars he had saved, he enrolled at The Pennsylvania State College, where he pursued the study of agriculture and soon became primarily interested in dairying. He found that room and board were expensive (board, $2.50 a week) so for the first year he prepared his meals in his room. He was fortunate in that the room was equipped with a steam radiator. From that radiator he attached a rubber hose to a double boiler arrangement (two tin cans) which made it possible for him to have "cooked" meals during the winter of 1905. He was given a highly-sought position as waiter in the college dining halls during his sophomore year, a position he kept until graduation.

In 1909 he graduated with honors and was the recipient of the John W. White Scholarship ($400) for graduate study. With this scholarship, he went to the University of Wisconsin, where he completed his Master of Science degree in 1910. He then returned to Pennsylvania, accepted a position at Penn State as assistant in dairy husbandry, married Jesse E. Canon, and settled down to start his professional career in the field of dairy science.

Young men of his ability and education were much in demand in those days, and in 1911 the University of Vermont convinced him that he should head its Animal and Dairy Husbandry Department. He served with notable success in that position for 4 years, at which time Penn State, a rapidly growing institution, asked him to return to be in charge of dairy husbandry extension. His four years in extension work, from 1915 to 1919, enabled him to become thoroughly familiar with the state's dairy industry and to become acquainted with its prominent personages. The following year he assumed the position of head of the Department of Dairy Husbandry and maintained it with distinction until his retirement in 1948.

During his senior year in college, he entered an essay contest in agriculture. His essay entitled "Improving the Dairy Industry in Pennsylvania" was adjudged first, and he received the Barlow prize of $100 (five $20 gold pieces). The subject of this essay was the model upon which he devoted his life to the dairy industry.

Professor Borland was always a very versatile and energetic worker, as well as an interesting speaker. When he became head of the Penn State Dairy Department, there was a staff of 4 men, a herd of 60 head of dairy cattle, a modest college creamery, and about 40 students studying dairying. During his tenure as head, the staff expanded to 15, a new modern dairy building was built, the herd increased to approximately 500 head, more than 1300 students were graduated, and a strong well-balanced research program was established. Because of heavy administrative and teaching responsibilities over the years, his personal research activities were limited to a few outstanding pieces of work in the field of nutrition. Two of these, "The Effect of Low, Medium and High Protein upon Production and Health of Dairy Cows" and "Input and Output Relationships in Milk Production" are particularly noteworthy. He was always a strong advocate of research, surrounded himself with outstanding research scientists, and continuously sought support for their programs.

As a teacher and counselor of men, he had few peers. His interest in students went far beyond the classroom, and many sought his advice long after graduation. His philosophy of education encompassed the "whole man" and placed much emphasis on character development and preparation for living as well as preparation for making a living. His students throughout the country hold him in high esteem and many credit their success to his friendly and sincere counsel.

Throughout his career, he worked closely with the industry of his state and was much sought after as a speaker and a judge of dairy cattle. He was active in the affairs of the Pennsylvania

Dairymen's Assoc. and served as its president from 1925 to 1927.

Professor Borland has always taken an active interest in the affairs of the American Dairy Science Association. From 1915 to the present, he has missed very few annual meetings of the association. Over the years he held many committee assignments and official positions in association affairs, serving as vice-president from 1920 to 1922, president from 1922 to 1924 and president of the Eastern Division of A.D.S.A. in 1925. He was a member of the Journal Management Committee from 1932 to 1944 and served as chairman, 1943 and 1944. In 1948 he was presented with the Certificate of Honorary Life Membership, a much deserved recognition for his service to the A.D.S.A.

From his college days, when he was president of the Penn State Christian Assoc., he has been active in church affairs. He was one of the founders of the Westminster Foundation at State College, Pa., and has been a trustee to this group since 1939. As an elder in the Presbyterian Church for many years, he has contributed much to his home community and has always had a keen interest in the spiritual welfare of students regardless of creed or church affiliation.

Among his many and varied activities, he has been a contributing editor of the Pennsylvania Farmer since 1923, was delegate to the 8th World's Dairy Congress, London, Reading, Edinburgh, and Glasgow in 1928 and judge of the 4-H International Dairy Cattle Judging Contest at Kent, England, in 1928. He is a member of a number of honorary and professional societies.

For a period after his retirement, he and Mrs. Borland traveled considerably, spending time with their children, Gerald C. Borland of Westfield, Pa., and Margaret E. Borland Hartzell of Redlands, Calif. However, they still make State College their home.

He has been ill since undergoing surgery in 1952, but his health is improving slowly, and he still maintains a keen interest in college, national, and world affairs. He follows the Journal closely every month and has expressed his great pleasure with the People and Events section. In spite of his illness and slow recovery, he still maintains his characteristic sense of humor. This was much in evidence at the recent A.D.S.A. meeting when in addressing the Penn State alumni group, he said: "Two years ago, the Old Man with the scythe just about caught me, but right now I'm gaining on him."

The industry is fortunate that men such as Professor Borland entered the field and devoted their lives to the training of young men and to the advancement of dairy science.

Michigan State College Hosts to A.D.S.A., 1955

Michigan State College at East Lansing, which will be host to the American Dairy Science Assoc., June 20-23, has become the "conference center" of the Midwest. More than 400 conferences, with a total attendance of 160,000, used the Michigan State facilities last year. These meetings ranged from technical courses for beekeepers, canning industry fieldmen, and medical technologists to conferences in the areas of safety, health, international relations, home and family living, agriculture, business, industry, labor, education, and public service.

The facilities and faculty of Michigan State College are made available to these conference groups through the operation of the Continuing Education Service. This service was established in 1948, and the word "continuing" recognizes that education never ends for anyone—it is a lifelong process.

A unique, seven-story, $2,000,000 building, known as "Kellogg Center for Continuing Education," serves as headquarters for C.E.S. Many of the panel programs, workshops, and other activities of the A.D.S.A. meeting will use Kellogg Center. The facilities of this attractive building, custom designed to fit the needs of an educational conference center, include 193 hotel-type rooms, each with private bath, dining facilities for more than 1,000 persons, an auditorium, 16 conference rooms, huge parking area, and other facilities.

To provide the needed facilities for the increase in conference activities since the construction of the Center in 1951, a half-million-dollar expansion program was started in January, 1955. The new additions will increase the conference registration lobby, enlarge the large ballroom-dining room, and add more conference rooms.

The conference activities also utilize many of the other buildings on the extensive M.S.C. campus. The A.D.S.A. annual meeting will have its headquarters in the Harrison Road

Many of the panel programs, workshops, and other activities of the A.D.S.A. meeting, June 20-23, at Michigan State College will use the unique, seven-story "Kellogg Center for Continuing Education."

Residence Halls, across the street from Kellogg Center. This residence hall development now includes three dormitories, the fourth building, Brody Hall, serving as the dining area. Brody Hall has a capacity to serve 3,000 in the many dining rooms.

Cooperative Graduate Summer Sessions in Statistics

The Univ. of Florida, North Carolina State College, Virginia Polytechnic Institute, and the Southern Regional Education Board are jointly sponsoring a series of cooperative summer sessions in statistics. The first of these sessions was held during the summer of 1954 at Virginia Polytechnic Institute. Attending were 89 students from 26 states and the District of Columbia and from India, Finland, Canada, Australia, China, Hawaii, and the Philippines. The second session will be held at the Univ. of Florida from June 20 to July 29, 1955. A session is scheduled at North Carolina State College in 1956 and at Virginia Polytechnic Institute in 1957.

The session will last 6 weeks and each course will carry approximately three semester hours of graduate credit. The program may be entered at any session, and consecutive courses will follow in successive summers. The summer work in statistics may be applied as residence credit at any one of the cooperating institutions, as well as certain other institutions, in partial fulfillment of the requirements for a master's degree. The catalog requirements for the degree must be met at the degree-granting institutions. Each doctoral candidate should consult with the institution from which he desires to obtain the degree regarding the applicability of the summer courses in statistics.

The faculty for the 1955 session will include: Professors R. L. ANDERSON, N. C. State College; D. B. DUNCAN, Univ. of Florida; BOYD HARSHBARGER, Virginia Polytechnic Institute; C. E. MARSHALL, Oklahoma A. and M. College; H. A. MEYER, Univ. of Florida; G. E. NICHOLSON, JR., Univ. of North Carolina; P. J. RULON, Harvard Univ.; W. L. SMITH, Univ. of North Carolina; and D. E. SOUTH, Univ. of Florida.

Courses to be offered this summer are: Statistical Methods I, Statistical Methods II (Design of Experiments), Statistical Theory I, Statistical Theory II (Inference and Least Squares), Advanced Analysis I, Theory of Sampling, Theory of Statistical Inference, Mathematics for Statistics, Statistical Research in Education and Psychology, and Seminar on Recent Advances in Statistics.

The total tuition fee is $35 for the 6-week term. The holder of a doctorate degree, upon acceptance, may register without the payment of any tuition fee. Inquiries should be addressed to PROFESSOR HERBERT A. MEYER, Univ. of Florida, Gainesville.

Pennsalt Announces Opening of New Plants

The Pennsylvania Salt Mfg. Co. opened a new plant at Delaware, Ohio, Jan. 18. This plant, Pennsalt's thirteenth production facility, is a component of the Company's Chemical Specialties Division. A similar installation nearing completion at Chicago Heights, Ill., was dedicated Feb. 10.

K. R. Johnson Joins Staff at Idaho

K. R. JOHNSON has accepted a position on the staff of the Dept. of Dairy Husbandry of the Univ. of Idaho. He will be in charge of instruction and research in dairy cattle breeding. He received his M.S. degree from the Univ. of West Virginia and his Ph.D. degree from Purdue Univ., where he did research and teaching in dairy cattle breeding.

Dr. Johnson replaces W. R. HARVEY on the Univ. of Idaho staff. Dr. Harvey has accepted a position as biometrician, in charge of the livestock research staff, Agricultural Research Service, USDA, Beltsville, Md.

Wyoming Holds Quality Meeting

T. D. HARMAN, acting secretary of A.D.S.A., was one of the speakers at the Univ. of Wyoming conference held Feb. 14-15. The theme of the program was "Plant Sanitation and Quality Products." Other speakers were W. H. MARTIN of Kansas State College, and W. A. HOSKINSON of Arden Sunfreze Creameries, Salt Lake City. Martin judged the dairy products show and contest.

D. H. Nelson of Massachusetts Dies

D. HORACE NELSON, 56, assistant professor of dairy industry at the Univ. of Massachusetts, died of a heart attack Jan. 14 at his home. He was born in Franconia, N. H., Oct. 14, 1898. He was a graduate of the Univ. of New Hampshire and received his M.S. from the Univ. of Missouri in 1922. From 1922 to 1937 he was a member of the faculty at the Univ. of California at Davis. He studied at Pennsylvania State College, where he received his Ph.D. degree in 1939. He was a staff member at New Mexico State College from 1939 to 1945, when he joined the faculty at the Univ. of Massachusetts.

Louisiana Attracts Graduate Students

The graduate program of Louisiana State Univ. now includes 10 students representing Pennsylvania, Oklahoma, Tennessee, and Louisiana universities.

M. W. SCHEIN has returned to his duties at L.S.U. after completing graduate work at Johns Hopkins Univ. School of Hygiene and Public Health. His present position is associate animal climatologist.

Creamery Package Appoints C. Y. McCown to New Post

C. Y. McCown has been appointed director of purchases and inventories for Creamery Package Mfg. Co., with headquarters at the firm's general offices in Chicago. "Mac" McCown joined the company in 1933 as a research engineer. In 1943 he was made assistant to the vice-president in charge of factories. His new duties carry the responsibility for direction and coordination of the purchasing of factory materials and control of factory inventories. McCown graduated from the Univ. of Illinois in 1930 with a B.S. in mechanical engineering. He received an M.S. in dairy husbandry from the same school in 1933.

Michigan State to Hold Dairy Engineering Conference

Michigan State's third annual Dairy Engineering Conference will be held March 8 and 9. Subjects to be covered include design of dairy plant floors, dairy waste treatment, equipment maintenance, manufacture of milk fat, milk vending equipment, refrigerated trucks, and cold sterilization of milk. The conference is sponsored by the Dept. of Agricultural Engineering.

North Carolina State College News

R. K. Waugh is taking a 2-year leave of absence from his duties as head of the Dairy Husbandry Section to assist with the agricultural improvement program in Peru, S. A. He will be stationed at an outlying experiment station at Tingo Maria, where he will work with the problem of improving the dairy industry. The work will be directed by R. W. Cummings, director of the Experiment Station, N. C. State College, who is also spending some time in Peru, coordinating the activities of various workers from the College, all of whom are participating in an agricultural improvement contract between the Peruvian Government and N. C. State College. J. E. Legates will be acting head of the Section during the absence of Dr. Waugh.

The Annual Dairymen's Conference was held on Feb. 15 and 16 at N. C. State College. Highlights of the conference were discussions on roughage production and utilization, bulk handling of milk, and selling milk through the American Dairy Assoc.

Kentucky News

A. W. Rudnick, Jr., instructor and assistant in dairy husbandry at the Univ. of Minnesota, has been elected to the staff of the Dairy Section at the Univ. of Kentucky, to fill the vacancy created by the resignation of E. N. Boyd. In his new assignment Dr. Rudnick will serve as superintendent of the processing plant, assistant professor of dairy technology, and assistant dairy technologist. His appointment is effective Feb. 1.

The board of directors of the Dairy Products Assoc. of Kentucky has announced the appointment of Frank Manning to the office of executive secretary, effective Jan. 1. He will fill the vacancy caused by the accidental death in June, 1954, of Fred Flynn. Mr. Manning has been associated with his brother, James Manning, in the operation of the H-B Milk Co. at Frankfort, Ky. Frank's previous business experience, his interest in dairying, and his personality all qualify him to fill this appointment in a creditable manner.

The Annual Fieldmen's and Herdsmen's Short Course was held at the Univ. of Kentucky on Feb. 21-23, 1955. This event is sponsored by the U.K. Dairy Section in cooperation with the Kentucky Cream Improvement Assoc., the Kentucky Manufactured Milk Assoc., and the Kentucky Purebred Dairy Cattle Assoc. The Kentucky Assoc. of Milk and Food Sanitarians will also meet in conjunction with the short course.

Idaho Short Courses

A short course in Starter Making and the Manufacture of Cottage Cheese will be held March 14-19 on the campus at Moscow, Idaho. Instruction will be given in the composition and properties of milk; milk quality tests; starter making, including bacteriology of starters, starter activity tests, and growth inhibitors; manufacture, defects, and keeping quality of cottage cheese; and the manufacture of some related varieties of cheese.

A course in Milk Plant and Dairy Farm Sanitation will be given March 21-26. This course will include instruction in the principles of sanitation, the composition of cleaning and sterilizing compounds, correction of water for proper cleaning, dairy farm sanitation, pipe line milkers, bulk handling of milk, and in-place cleaning of dairy equipment on the farm and in the plant.

Oregon Considers Milk Control Measures

The Oregon Dairyman's Assoc. annual meeting was held at Bend, Ore., Jan. 12-14 with about 150 persons in attendance. Promotion and advertising of dairy products and dairy legislation headlined the meeting. Twenty-one resolutions were passed. There was considerable discussion on a resolution to recommend state legislation to control producer prices on milk before the resolution was voted down. Milton Rider, outgoing president, had earlier expressed the opinion that the dairy industry in Oregon should not again put itself in a position where it could be attacked by the voting public. He

suggested that either control within the industry itself or a federal marketing order would be preferable to a new state law. It was pointed out that legislation is being prepared by certain elements of the industry to be submitted to the Oregon legislature now in session.

Ohio Events

T. KRISTOFFERSEN has been appointed research associate in The Ohio State Univ. Research Foundation. Dr. Kristoffersen, a native of Denmark, recently received his Ph.D. degree in dairy bacteriology at Iowa State College. Under a grant from the Diversey Corp. of Chicago, he is making a study of sanitizers for the dairy field. K. K. Fox was appointed as instructor, and C. V. MORR as assistant instructor. Both are working on advanced degrees in dairy technology.

IWAO SATO, Sapporo, Japan, is working toward a master's degree in dairy technology. He is following in the footsteps of his father, who received his M.S. degree from The Ohio State Univ. in 1921.

A Milk Sanitarians Short Course will be held March 14-18. Major topics of discussion include principles of inspection, law interpretation, and sanitation. This course is sponsored by The Ohio State Univ. in cooperation with the state Departments of Health and Agriculture.

Minnesota News

J. H. KOSHI has accepted a teaching and research position on the dairy staff at the University of Hawaii.

DAVID ARNOTT has been granted an assistantship at Pennsylvania State Univ.

CLYDE HURRELL has returned to New Zealand after completing work for his M.S. degree.

FRED FOREMAN has accepted an assistant professorship on the dairy husbandry staff at Iowa State College.

VERNON BALDWIN, formerly research fellow at the Univ. of Minnesota, is now associate professor in the Dairy Department and associate dairy husbandman in the Extension Service at Virginia Polytechnic Institute. He will be in charge of the project on dairy cattle improvement through breeding.

V. H. TOWNLEY, research associate, died on Jan. 1 after an illness of 2 weeks.

A new dairy science scholarship of $1,500 will be awarded to a qualified student within the next few months for graduate study in dairying at the Univ. of Minnesota. The scholarship will be known as the John Brandt Memorial Scholarship and was recently created by the John Brandt Memorial Foundation. Mr. Brandt was president of Land O' Lakes Creameries in Minneapolis at the time of his death.

E. M. (BILL) BARKER, at present research and quality control director for the Rochester Dairy Coop. at Rochester, Minn., will resign March 1, according to CHARLES STUBE, general manager. Mr. Barker will be associated with the Darimix Control Co., LaCrosse, Wis. KERMIT MADISON will be promoted from laboratory supervisor to laboratory director.

News from Here and There

TEMPLE MCALLISTER, an Ohio dairyman, has been convicted for criminal contempt and fined $2,000 in the Dauphin County Court of Pennsylvania for violation of the state's milk pricing law. The offense was charging a deposit on jugs of milk and redeeming the deposit with an extra dividend. This, in effect, was a reduction of the retail price of milk, which is fixed by state law in Pennsylvania.

Attorneys for the Federal Trade Commission have appealed the dismissal of complaints against the Blanton Co., St. Louis, and the Reddi-Spred Corp., Philadelphia, by examiners who held that those firms had not violated the Margarine Act of 1950, which prohibits advertising suggesting that margarine is a dairy product. The principal part of the examiners' findings forming the basis of the appeal is that dairy ingredients, such as milk, cream, and butter, could be emphasized in advertising independently of the other ingredients present.

According to a survey made by the *Minneapolis Star and Tribune*, 45% of the families in cities, 43% in small towns, and 29% of the farm families in Minnesota use evaporated milk. The use of dried milk was reported as about 40% of that of evaporated milk.

A new slogan has been coined for the butter industry by ADA—"My mom's gone back to butter." Some milk people are also reviving the old slogan, "Put the milk pitcher back on the table." Some thought might be given to reviving the ice cream slogan, "Serve it and you please all."

Additional acquisitions by Foremost Dairies, Inc., include Emmadine Farms, Inc. in Westchester Co., N. Y., distributors of ice cream and milk, and Slade's Dairies, Inc., of Albuquerque, N. Mex.

Recent mergers announced by Beatrice Foods Co. include Greenbrier Dairy Products Co., of Beckley, W. Va., and Russell Creameries, of Superior, Wis. HUGH HUTCHISON is general manager of the Greenbrier plant. He is national chairman of June Dairy Month and is president of the Intern. Assoc. of Ice Cream Manufacturers.

T. G. MONTAGUE, president of the Borden Co., New York City, has been elected a director of the Greenwich (Conn.) Trust Co. He is also a trustee of the Bank of New York and the Northwestern Mutual Life Insurance Co. and is a director of the American Sugar Refining Co. of New York.

The Louisiana Dairy Products Assoc. held its annual meeting in New Orleans Jan. 16-18. BRUCE BALDWIN, vice-president of Abbotts Dairies, Inc., and president of the Milk Industry Foundation, and ROBERT HIBBEN, executive secretary of the Intern. Assoc. of Ice Cream Manufacturers, were featured speakers.

The Taylor Freezer Corp., of Rockton, Ill. has changed its name to Flavor Fast Foods, Inc., according to an announcement recently made by its president, L. A. M. PHELAN. The company will continue to manufacture its Taylor freezer line.

MORRISON LOEWENSTEIN has resigned his position with the Oklahoma A. & M. College Dairy Husbandry Dept. to become director of research for the Crest Foods Co., Ashton, Ill.

A Tribute to Vitamin D Milk

At the recent annual meeting of the Wisconsin Milk Dealers' Assoc. the following resolution was unanimously adopted.

"WHEREAS, this year marks the 25th anniversary of the discovery of the process for the addition of vitamin D to foods, and

"WHEREAS, milk has long been regarded, during this period, as the ideal food to which vitamin D should be added, and

"WHEREAS, the early acceptance by the medical profession and by the milk industry followed this discovery, and

"WHEREAS, during the ensuing years the process of adding vitamin D to homogenized milk has come to be almost universally accepted and universally used by the milk industry, and

"WHEREAS, the result of this prompt acceptance by the dental and medical professions and by the milk industry of this important scientific fact has resulted in incalculable benefits to the health and well being of our entire population—particularly growing children, and

"WHEREAS, in the instance of growing children, rickets was a common illness among a large percentage of children prior to the fortification of milk with vitamin D, and

"WHEREAS, it is now a fact that there has been almost a complete disappearance of rickets among children, a fact to which vitamin D fortification of milk is entitled to a great deal of credit,

"THEREFORE, be it resolved that the Wisconsin Milk Dealers, in convention assembled reaffirm their confidence in the process of adding vitamin D to milk and urge that all milk dealers everywhere continue to fortify all homogenized milk with vitamin D in the amounts which have been determined to be adequate and to emphasize to their customers, to their employees, and to the public at large the importance of the vitamin D fortification of milk."

Completed Theses

M.S. Degree:

D. R. L. ARNOTT—The effect of certain chemical factors on the melting quality of process cheese. Univ. of Minnesota.

C. O. HURRELL—A survey of consumer-packaged nonfat dried milk solids. Univ. of Minnesota.

Sc.D. Degree:

M. W. SCHEIN—Group behavior patterns in dairy cattle and their effect on production. Johns Hopkins Univ.

Ph.D. Degree:

P. J. DZIUK—Morphological changes in the bovine endometrium during normal cycles and following attempts at synchronization of estrous cycles with progesterone. Univ. of Minnesota.

J. H. KOSHI—Complementary milk and its relationship to lactation. Univ. of Minnesota.

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Newer Concepts of Dairy Waste Disposal[1]

R. H. Jackson and G. H. Watrous, Jr.

Department of Dairy Science, The Pennsylvania State University

It has been said many times that the best solution to the waste disposal problem in the dairy industry is the control of losses resulting from manufacturing processes. Regardless of how often this fact has been restated, it is of utmost importance to the industry and cannot be over-emphasized, because it has a two-fold significance. First, these losses represent a monetary investment going down the drain; secondly, they represent a disposal problem. This article is not concerned, however, with the prevention of such losses but rather with the treatment of dairy wastes after they have entered the sewers of the plant.

In the past, very little attention was given to the liquid waste which was discharged by plants engaged in manufacturing dairy products. Frequently a stream was near by, into which the dairy could conveniently discharge its waste. Today, a large population and numerous industries make it difficult to send waste materials down-stream without causing repercussions. With increasing emphasis on the treatment of industrial wastes, and campaigning by fishing enthusiasts and wildlife groups for cleaner streams, it is necessary for those associated with processing milk and milk products to consider or provide adequate treatment for the waste from their plants. However, the mere provision for treatment is not sufficient. Too often a disposal unit will be installed only to satisfy state or federal demands. Frequently, such units are poorly designed and provide inadequate treatment, which eventually leads to dissatisfaction on the part of all concerned.

Biological Oxidation of Dairy Wastes

In the process of oxidizing dairy waste biologically, the essentials are a healthy bacterial culture to decompose the milk solids in the waste and at least 0.5 p.p.m. of surplus dissolved oxygen to maintain aerobic conditions. In this sense, a healthy bacterial culture is one which is multiplying rapidly at approximately a pH 7.0 and a temperature of 90° F. The maintenance of the minimum of 0.5 p.p.m. of surplus dissolved oxygen in the system is the key to efficient treatment. Many treatment plants are discharging inadequately treated waste with offensive odors because the amount of dissolved oxygen falls below the required minimum. In some cases the volume of air put through the system is sufficient, but the air dispersing device is inefficient. There are many types of dispersing devices having an efficiency range of 2 to 25% in dissolving atmospheric oxygen in the liquid of a waste disposal system. The amount of air which must be pumped through a system will depend upon the efficiency of the air dispersing device in making enough dissolved oxygen available for the concentration of bacteria which must be maintained to handle the influent or incoming waste loads. The cost of treatment will be proportional to the biochemical oxygen demand (B.O.D.) of the waste to be treated and the efficiency of the air dispersing device.

The basic reaction for milk waste assimilation is as follows:

Milk solids (1.0 lb.) + bacterial cells (1.0 lb.) + oxygen (0.45 lb.) = bacterial cells (1.5 lb.) + CO_2 (0.45 lb.) + H_2O (0.5 lb.)

As the equation indicates, the products of bacterial decomposition are the same bacterial cells plus the formation of new cells equal to 50% of the milk solids, and also carbon dioxide and water. In other words, 50% of the milk solids is transformed into new cells and the remainder, principally lactose, is utilized by the bacteria for energy. The protein is converted into new cell material, which offers the sludge organisms a chemical structure empirically similar to their own. Since lactose contains no nitrogen, it cannot be used as a source of new bacterial cells. Experimental evidence indicates that the ratio of protein to lactose in milk solids offers a balanced diet for a reproducing bacterial population, and any serious deviation from the ratio of 51% lactose to 37% protein will interfere with metabolic activity and cell reproduction. Unfortunately, dairy waste does not always have an ideal lactose/protein ratio.

The waste from many plants, especially those discharging whey from large cheese operations,

[1] Authorized for publication, October 30, 1954, as Paper No. 1915 in the Journal Series of Pa. Agr. Expt. Sta.

is not suitable to biological oxidation unless extra precautions are taken. Cheese whey is low in protein and high in lactose concentration. Normal milk solids contain approximately 37% protein and whey solids contain 13%; lactose in whey solids exceeds lactose in skimmilk solids by 20%. Whey solids, therefore, have an insufficient amount of protein available for conversion to new cells. Whey, added in large quantities as it would be from a cheese plant, furnishes more lactose than can be used by the limited bacterial population for energy and consequently much of it remains in the treatment plant in solution, eventually being discharged in the effluent. If whey is to be treated aerobically, a nitrogenous supplement must be added to accelerate bacterial multiplication. Possibly the cheapest and most practical supplement is agricultural ammonium sulphate. Twenty-two pounds of this chemical supplies the nitrogen deficiency in 1,000 lb. of whey. This will supply the equivalent of approximately 30 lb. of milk protein and should be added directly to the oxidation tank prior to the addition of whey. This discussion, of course, asumes that an active bacterial culture exists. To start a culture for an aerobic waste disposal system for a cheese plant, it is best first to acclimatize the bacteria to a milk solids diet before switching over to whey with an added nitrogen supplement. It is doubtful if a culture could be started on merely whey and a supplement as described.

Disposition of Alkali Wash Water

Another consideration with this type of biological waste disposal system is the problem of disposing of bottle-washer alkali and similar products. If the solution from the bottle washer passes directly down the sewer into the waste disposal unit, it will usually raise the alkalinity to pH 10, or higher, and destroy the bacteria necessary for the oxidation of the milk waste. The best solution to this problem would be the construction of a small seepage pit into which such material could be discharged when necessary.

There Is No Sludge Problem

Fortunately, there is no sludge accumulation problem. The bacterial population maintains a level which is proportional to the amount of waste material it encounters. This is accomplished by endogenous respiration or the auto-digestion of the bacteria. When the sludge organisms have no milk solids to decompose, they begin to digest themselves, thereby eliminating the necessity of sludge removal.

Waste Disposal System at the Penn State Creamery

Two years ago, The Pennsylvania State University agreed to a request by the U. S. Department of Agriculture to undertake a research project in developing design specifications which could be used by dairy plant operators to economically construct and efficiently operate a waste disposal system suited to their individual needs. This project utilized the material, personnel, and cooperation of the University's Departments of Dairy Science and Sanitary Engineering. Experimentation has been carried on with a 20,000 gal. wooden tank to receive a portion of the daily waste from the University Creamery. Two 5 h.p. positive displacement pumps force air into the tank through eight #7 Penberthy ejectors, mounted through the wall around the circumference of the tank. Only one of these pumps operates during the 8-hour loading period from 8 A.M. to 4 P.M., during which time the oxidation tank receives 10,000 gal. of waste. The other pump, normally unnecessary, was installed to supply additional oxygen when experiments with increased quantities of air were being conducted. The pilot plant is almost completely automatic and possesses an over-abundance of equipment, which would not be necessary on a commercial installation.

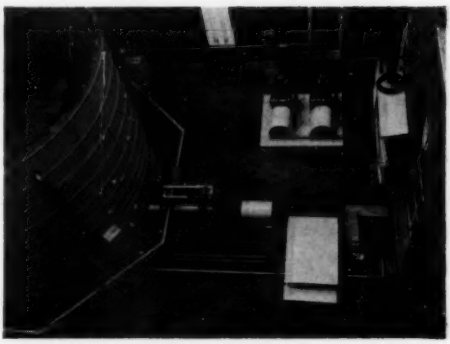


FIG. 1. An overhead view of the pilot plant prior to the initial waste load.

During a typical daily operation, 5,000 gal. of "seed" from the previous day is retained in the oxidation tank. To this is added 10,000

FIG. 2. A closer look at the installation showing the two blowers, recycle pump, manifold piping, and one of the Penberthy ejectors (lower left).

gal. of influent which is representative of the Creamery's waste. Two centrifugal pumps, operating in a sump, are activated by automatic time switches and begin loading the oxidation tank with influent at 8 A.M., in order to obtain the waste from the morning operations. A recycle pump and an air pump or blower also begin to operate at this time. Recycling is a means of aspiration and this alone can maintain aerobic condition in the tank when no waste load is being added. In addition to this, recycling keeps the tank's contents thoroughly agitated. At 4 P.M. the blower stops, with recycling and aspiration continuing until 3 A.M., at which time the recycle pump automatically stops. Settling of bacterial cells, protozoa and any nonoxidizable material occurs, and at 5 A.M., 10,000 gal. of clear effluent is automatically discharged. This effluent is equal in volume to the influent, thus making this a batch-type operation. The capacity of the oxidation tank must be proportional to the volume of waste to be treated.

It has been found that optimum temperature and pH is easily maintained. The average tank temperature has approximated 90° F., and the reaction usually remains close to pH 7.0. The buffering action of bacterial proteins, coupled with addition of acids and alkalies from daily operations, has resulted in a nearly neutral solution. Under conditions of this study, it has been possible to obtain a 95% reduction of the B.O.D. of the influent without difficulty.

Although much research is needed to complete our knowledge of the biological oxidation of dairy waste, it is possible, with the facts obtained in this study, to design a system of this type that will operate successfully.

The Value of the Coliform Test Applied to Fruit Flavored Ice Cream

Care Must be Exercised in Interpretation of Results

F. W. Barber

National Dairy Research Laboratories, Inc., Oakdale, Long Island, N. Y.

Coliform organisms as described by *Standard Methods for the Examination of Dairy Products* are "all aerobic and facultative anaerobic Gram-negative, nonspore-forming bacteria which ferment lactose with gas formation." They are normally present in raw milk, water, soil, and feeds and on the hands, but except in rare instances they are destroyed by proper pasteurization treatments. Therefore, their presence in a pasteurized dairy product is an indication of contamination after pasteurization by contact either with improperly cleaned and sanitized equipment or with raw ingredients.

The value of the coliform test on milk products has been well established as a quality control measure and as a technique of importance in the evaluation of sanitization and sterilization procedures in the dairy plant. Recent studies on coliform organisms in ice cream have indicated that the presence of these organisms in frozen desserts has the same sanitary significance as does their appearance in pasteurized milk. Although the significance of coliform organisms in milk and in frozen desserts may be the same, the interpretation of coliform test results on frozen desserts is more complicated. With milk or cream only a single ingredient is involved, but with ice cream numerous ingredients are used and each has its own typical bacterial flora. Fruits, nuts, and flavoring materials have at times been found to have a high total count as well as a high coliform count.

The proper use of the coliform test makes it possible to check each ingredient and follow each processing step through the plant operation. It indicates at which point attention is needed in processing, cleaning, and sanitization of equipment, or in the bacteriological condition of ingredients. Thus, the coliform bacteria are good indicator organisms in the control of quality.

The test is further complicated by the fact that in ice cream lactose is not the only sugar present in the product. Sucrose is also added to ice cream and may cause the development of false positive coliform tests unless confirmatory tests are carried out. A recent investigation on the coliform problem in fruit ice cream can be cited as an example of the value of the coliform test as applied to fruit ice cream.

A survey of the literature and actual plant data indicated that in the coliform testing of ice cream frequently fruit ice cream showed coliform counts in excess of 10 per gram. Further investigation showed that in the majority of cases these high coliform counts were obtained on strawberry, peach, and banana ice cream. It was reasoned that these high counts could be caused either by coliform contamination within the plant during processing or by a high coliform content in the fruit puree. A high fruit puree count could be due to either a natural coliform flora on the fruit itself or a contamination build-up during the fruit processing operation.

As these studies progressed it was soon obvious that the positive coliform tests noted on strawberry, peach, and banana ice cream were

not due to coliform contamination within the ice cream plant during processing. Line runs indicated that both the fruit puree and the plain mix could be free of any coliform organisms but when these two ingredients were mixed together, positive coliform reactions were obtained. Further studies disclosed that proper cleaning and sterilization of the fruit injector and the freezer did not eliminate the positive tests.

Red Colonies May Be Nongas-Forming

In addition, it was observed that DLA (desoxycholate lactose agar) plates of fruit puree or fruit juice often contained many small red or pink colonies. Some of these colonies could be easily mistaken for coliform colonies, especially when it is realized that smaller colonies develop on crowded plates. These colonies were picked into lactose broth tubes and found not to form any gas from this sugar. The colonies developing on DLA plates of fruit ice cream also were isolated—and in many instances these produced no gas from lactose.

In the development of the coliform test procedures it was recognized that false positive tests might be obtained on samples that contained carbohydrates other than lactose. In fact, *Standard Methods* states that under these circumstances when positive presumptive tests are obtained, further confirmatory tests should be made. In these studies it was recognized that false positive results were being obtained on the samples of fruit ice cream. This in itself was not a serious problem, since according to *Standard Methods* the positive presumptive tests on ice cream should be confirmed before results are reported as positive coliforms. The problem arose from the fact that often laboratories, both of the dairy industry and of regulatory officials, relied only on solid media tests for coliform organisms, and hence false positive coliform results on fruit ice cream might be reported. It was noted later in these studies that the same false positive results could be obtained with liquid media tests.

Sucrose a Factor in Growth of False Positive Coliform Organisms

In an attempt to learn more about these false positive coliform tests on fruit ice cream, additional studies were initiated to investigate the source of the responsible bacteria. The bacteria involved are for the most part Gram-negative short rods, although some Gram-positive cocci and a few yeast have also been encountered. All the organisms are indistinguishable from true coliform bacteria in solid media tests and sometimes in liquid media tests in the presence of ice cream solids. The small amount of ice cream solids carried over to the plate or the tube when 1 ml. or even 0.1 ml. of ice cream is tested is sufficient to result in these false positive reactions. Further tests have shown that it apparently is the sucrose which is responsible for the development of the characteristic large red colonies on the solid test media and the production of gas in the liquid test media.

The small red or pink colonies noted on DLA plates of fruit juice or puree were isolated and replated on DLA and DLA with added sucrose (1%) or added ice cream mix. The colonies which then developed on the special media were large and deep cherry-red in color, indistinguishable from "true" coliform colonies, while on the regular DLA medium the colonies were still the original small red or pink colonies. True coliform organisms (those producing gas from lactose) did not change in size or color with the addition of sucrose or ice cream solids to the medium.

Bacteria isolated from various sources including raw fruit, processed fruits, fruit ice cream, etc., were typed by their growth characteristics on special media as shown below:

Type	Colony characteristic on DLA	DLA + Sucrose	DLA + ICM
A	small pink	large red	large red
B	small pink	small pink	small pink
C	large red	large red	large red
D	large red	large red	large red
X	no growth	medium red	medium red

Types A, B, and X showed no gas from lactose and might or might not show gas in the presence of added sucrose or ice cream mix. Type C showed gas from lactose and was considered a typical true coliform organism. Type D gave a colony characteristic similar to a true coliform but did not show gas from lactose. In the presence of sucrose or ice cream mix gas might or might not be produced. The isolant most frequently observed was Type A. All of these types were Gram-negative short rods, and preliminary identification tests have shown conclusively that these organisms, except Type C, do not belong to the coliform group.

Fresh Fruit Source of the False Positive Coliform Organisms

The original source of the false positive coliform organisms appeared to be the fresh fruit. Isolations were made from the raw fruit of strawberries, peaches, and bananas. They also have been found throughout the fruit processing operations and in the frozen packed fruit. There is some evidence that there may be a decrease in the numbers of these organisms in the frozen product upon prolonged frozen storage. The incidence of false positive coliform reactions seemed to be lower during the winter months in strawberry ice cream when the berries had been held in cold storage for prolonged periods before use in the ice cream.

These studies have shown that on fruit ice cream positive coliform tests can be obtained which do not indicate faulty pasteurization or improper cleaning or sanitization of equipment or contaminated ingredients. The test results must be confirmed and properly interpreted. If this is done, the value of the coliform test is not impaired.

The coliform test on fruit ice cream is of value in the determination of the bacteriological quality of ingredients, in the evaluation of the effectiveness of the cleaning and sanitization operations, in checking the efficieney of processing, and as a control on the general quality of the finished product. However, because of the normal flora of fruits added to the pasteurized mix and the presence of sugars other than lactose in the ice cream, confirmatory coliform tests must be made before positive coliform results are reported. When the need for care in the interpretation of coliform test results is recognized and followed, the value of the test on fruit ice cream is as important as its value in the testing of other dairy products.

VOLUME XXXVIII FEBRUARY, 1955 NUMBER 2

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

105. Comparison of the ring, plate, and whey tests for brucellosis, using milk from cattle of proven Brucella status. K. OGONOWSKI, Ministry of Agr. and Fisheries, Vet. Lab., Weybridge, and A. MCDIARMID, Agr. Research Council, Field Sta., Compton. Vet. Record, **66**, 48: 751. 1954.

Cattle used were Ayrshires and Ayrshire Crosses that are on a long-time experiment to study the degree and duration of immunity afforded by Strain 19 vaccine to a challenge dose of *B. abortus*. Thus, samples were available from the 1st and 2nd lactations of animals that were challenged and not challenged in groups vaccinated at various times before breeding age, and from non-vaccinated controls. Milk samples were tested at weekly intervals for 10 wk. after calving and the ring, plate, and whey agglutination tests for detecting *B. abortus* agglutinins in milk were compared. Authors considered the plate test most satisfactory from standpoint of accuracy and ease of application. Vaccination with Strain 19 prior to breeding age did not significantly influence appearance of agglutinins detectable by these tests.

R. P. Niedermeier

106. Hypomagnesaemia in cattle. R. ALLCROFT, Ministry of Agr., Vet. Lab., Weybridge. Vet. Record, **66**, 517. 1954.

Author briefly reviews the history of work on the aetiology of hypomagnesaemia in the 20 yrs. since the condition was first diagnosed. Recent work at Weybridge is discussed in detail. Recent work showed that feeding or drenching animals with 2 oz. of magnesium oxide daily increased serum Mg values from subnormal to normal levels and maintained values in the normal range during a period when hypomagnesaemia occurred in untreated controls. In one trial the animals did not respond following oral administration. No explanation for these conflicting reports was possible. However, on the basis of all observations the author advises the use of Mg rich supplements orally as a preventive and as a follow-up to the usual parenteral treatment of clinical cases.

R. P. Niedermeier

107. An experimental study of the control of Streptococcus agalactiae infection in dairy cattle. I. DAVIDSON, G. SLAVIN, and P. STUART, Vet. Lab., Ministry of Agr. and Fisheries. Vet Record, **66**, 33: 466. 1954.

This experiment was designed to check on the progress of *S. agalactiae* infection in a herd, with emphasis on practical means of limiting the natural spread of the infection. The laboratory dairy herd consisted of 9 sets of monozygous twins, all in their 3rd lactation, and none had been previously infected with *S. agalactiae*. The twins were divided into 2 groups, arranged opposite each other in the milking shed. Four artificially infected donors (nontwins) were introduced into the herd. Milking order was so arranged that the twin in one group was exposed in the milking routine (donors were milked first) in the same order and after the same donor as its twin in the other group. A steam sterilized set of teat cups, long and short milk tubes, pulsation tubes, and claw was used for each cow in Group A. The milking units were transferred from cow to cow in Group B without any disinfection.

Milk samples, teat swab samples, hand washings of milkers and water from the udder cloth bucket were checked weekly for presence of *S. agalactiae*. The length of the experiment was 16 wk. One cow in Group A became infected in 2 quarters, one of which showed mild, transient clinical mastitis. In Group B, 11 quarters of 5 cows became infected. Three quarters showed subacute and 3 showed chronic clinical signs of mastitis. The difference of the incidence of infection in the 2 groups was significant at the 5% level.

R. P. Niedermeier

108. The effect of oxytocic principle on experimental Streptococcus agalactiae mastitis in cows. I. H. PATTISON and I. M. SMITH. Vet. Record, **66**, 47: 724. 1954.

In a previous paper the authors reported the absence of benefit from the use of oxytocin in the treatment of *S. agalactiae* mastitis produced experimentally in goats. These results were contrary to the report (1953) that oxytocin was of value in treatment of mastitis in cows. This paper describes the authors' experiments

designed to measure the value of oxytocin in the treatment of experimentally produced *S. agalactiae* infection in cows. Eight Ayrshire cows in 4th mo. of lactation were used. Cows were paired and one quarter of each cow was experimentally infected. Oxytocin treatments (20 units intravenously) were given one of each pair of cows at 4, 8, 9, and 15 d. following infection. One wk. after the oxytocin treatment the infected quarters of each pair of cows were treated with oxytocin + penicillin and penicillin alone. The use of oxytocin alone or with penicillin showed no beneficial effects.

R. P. Niedermeier

109. Studies on bovine mastitis. I. Incidence of mastitis in cows and buffaloes. T. NARAYANAN and K. K. IYA. Indian J. Dairy Sci., **6**, 169. 1953.

The incidence of infection in Girs and crossbred animals was highest. A higher incidence of staphylococcal infection was found in cows, whereas in buffaloes the streptococcal infection was more prevalent. The streptococci were classified as *S. agalactiae, S. dysgalactiae, S. uberis,* and *S. pyogenes,* while the staphylococci were identified as *M. pyogenes* var. *aureus* and *M. pyogenes* var. *albus.*

W. C. van der Zant

BOOK REVIEWS

110. The Biochemistry of Semen. T. MANN. (Methuen & Co., London), John Wiley & Sons, New York. 240 pp. $2.90. 1954.

The first three chapters deal with morphological and physical properties of semen and the effect of various external factors on spermatozoa. Aside from this introductory material, the book is devoted to biochemical constituents of semen, and chapters are included on nucleoproteins, protein constituents and enzymes, lipids, fructose, the bases in semen, and citric acid and inositol. In general, the biochemical aspects of semen are ably and concisely presented. The author has done very well in assembling information on a rather wide range of compounds and in pointing out the similarities and differences between species. Many of these data are presented concisely in tabular form. Information on the lesser-known as well as the major constituents is well documented and presented in interesting fashion.

Some readers probably will not agree with the author on the relative emphasis, or lack of it, placed on certain subjects. For example, hydrogen ion concentration and buffering capacity is presented in about one and one-half pages, whereas other topics of perhaps lesser importance are allotted entire chapters. Those interested solely in practical artificial breeding will find only a few pages devoted specifically to diluents and associated problems, although essentially all the material included in the book has at least a potential relationship to the problem of semen preservation.

Research workers and students in this rather specialized subject matter field should find the book a valuable reference to information pertaining to the chemical constituents of semen and their possible significance. Particularly welcome are the numerous references to research conducted prior to 1900 which frequently are not available or are overlooked by investigators of the present era. R. J. Flipse

BUTTER

111. Status of the churn situation. D. C. ROAHEN, Creamery Package Mfg. Co., Chicago, Ill. Milk Products J., **45**, 12: 21. 1954.

Changing demands of present-day markets make it necessary to give some thought to what lies ahead in butterfat requirements. The butter industry must equip themselves so that they are not limited to the production of just one product. A versatile production unit is required which can produce the product most in demand for the current market conditions whether it is butter, anhydrous fat, or frozen cream. J. J. Janzen

112. Keeping quality of ghee. II. Effect of storing butter and ghee on vitamin A content. K. R. LALITHA and N. N. DASTUR. Indian J. Dairy Sci., **7**, 1. 1954.

Ghee was prepared by heating butter to 115° C., cooling and filtering through muslin. Approx. 44-82% of the vitamin A was lost by exposing the ghee to mild diffuse light for 14-24 d. When stored at room temp. in the dark with a small air gap in the container the loss in vitamin A after 120 d. of storage amounted to 12.5-36.1%; whereas under similar conditions after 85 d. at 42° C. the loss increased to 72-100%. W. C. van der Zant

CHEESE

113. A bacteriological study of cottage cheese with particular reference to public health hazard. P. R. LYONS and W. L. MALLMANN, Dept. of Bacteriol., Mich. State Coll., East Lansing. J. Milk and Food Technol., **17**, 12: 372. 1954.

The presence and survival of bacteria that may have public health significance was studied on 150 samples of market cottage cheese obtained from eight manufacturers. A large number of the cheese samples contained 220 and more coliform bacteria per 100 g. of cheese. The pH of cottage cheese is not sufficient to destroy coliform organisms. The authors conclude that cottage cheese may be considered a potential public health hazard and that unsatisfactory conditions exist in the manufacturing and packaging of cottage cheese.

H. H. Weiser

114. The utlization of Egyptian ewe's milk in the manufacture of Danni cheese. I. SIRRY. Indian J. Dairy Sci., **7**, 25. 1954.

The preparation and some of the characteristics of Danni cheese are discussed. Six % salt was added to the milk which contained 7.3% fat. Coagulation with rennet took place at 95° F. The curd was put in rectangular shaped molds on straw mats and was drained for 3 d. The cheese then was ready for consumption or pickling. The average fat content of the fresh cheese was 18.02%.

W. C. van der Zant

CONDENSED AND DRIED MILKS; BY-PRODUCTS

115. Process for the treatment of whey. B. DE GOEDE (assignor to Nederlandse Centrale Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek). U. S. Patent 2,695,235. 6 claims. Nov. 23, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 4: 784. 1954.

A product is formed having the foaming and heat coagulating properties of egg white by chilling liquid whey to cause precipitation of part of the lactose, adjusting the pH to 11-12, then acidifying to pH 9-10.5 to permit removal by filtration or other means, of the crystallized lactose and coagulated solids which form, leaving the desired protein product in solution.

R. Whitaker

DAIRY BACTERIOLOGY

116. Differentiation of growing bacteria and nongrowing bacteria by a staining technique. H. E. WADE and D. M. MORGAN. Nature, **174**, 4437: 920. 1954.

The technique is based on the fact that growing bacteria are more strongly basophilic than nongrowing bacteria. The culture was acidified with conc. HCl to give a conc. of 0.33N HCl. After centrifuging, the cells were washed in 0.33N HCl, diluted with a little water and treated with IR400 (OH) Resin to pH 3.5-6.0. An air dried and heat fixed slide was prepared and stained at 45° C. for at least 45 min. with 0.5% toluidine blue made up in 0.05M formate buffer at pH 3.5. The slide was then washed, blotted, and dried. It then was stained for 30-60 min. with 0.05N acetic acid saturated at room temp. with water soluble, yellowish eosin, washed with water, blotted, and dried. Cells stained blue violet are growing and cells stained pink are nongrowing. The use of this technique in determining the viability of bacterial suspensions was considered. R. Whitaker

117. The coliform bacteria of strawberries. J. O. MUNDT, G. A. SHUEY, and I. C. MCGARTY, Dept. of Bacteriol., Univ. of Tenn., Knoxville. J. Milk and Food Technol., **17**, 12: 362. 1954.

Gram-negative bacteria indigenous to plants and to the soil are usually present on strawberries used for processing and freezing. The natural sugar content of berries usually is sufficient to cause coliform-like organisms to produce gas. Washing the berries in chlorinated water or water containing detergents does not eliminate these microorganisms.

Paracolobacterium intermedium, considered indigenous to soil, largely was responsible for gas production in the presumptive media. The authors recommend lauryl tryptose broth for the presumptive test and suggest that positive tests be confirmed as to species identification.

H. H. Weiser

118. Relation between reduction times and plate counts of milk before and after pasteurization. C. K. JOHNS, Dept. of Agr., Ottawa, Can. J. Milk and Food Technol., **17**, 12: 369. 1954.

A comparative study of plate counts, methylene blue and reasazurin (triple reading) reduction times on milk before and after laboratory pasteurization has been made. The reduction methods showed a higher correlation with the plate count at 32° C. during the warm mo.

None of the milk samples with 30,000 thermoduric bacteria per ml. showed reduction times in warm months in excess of 3 hr. for resazurin or 5½ hr. for methylene blue.

H. H. Weiser

119. The effect of the incubation time and temperature on the determination of psychrophilic bacteria in milk by the agar plate method. J. C. BOYD, C. K. SMITH, and G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing. J. Milk and Food Technol., **17**, 12: 365. 1954.

The psychrophilic bacteria counts were higher in the samples incubated at 10° C. as compared to 5° C. Counts on milk samples stored at 5° C. for 10 d. were not obtained in less than 20 d. when the plates were incubated at 5° C.

H. H. Weiser

120. Coliform densities in pasteurized dairy products. J. ARCHAMBAULT, Quebec Ministry of Health, Montreal. J. Milk and Food Technol., **17**, 11: 342. 1954.

Coliform organisms in dairy products were estimated by use of violet red bile agar, B.B.L. agar, and brilliant green bile broth. The broth medium gave more consistent results than the solid media for detecting coliform bacteria in pasteurized samples. The potential sensitivity of this test is almost unlimited.

H. H. Weiser

DAIRY CHEMISTRY

121. Direct ethylenediamine tetraacetate titration methods for magnesium and calcium. C. W. GEHRKE, H. E. AFFSPRUNG, and Y. C. LEE, Dept. of Agr. Chem., Mo. Agr. Expt. Sta., Columbia. Anal. Chem., **26**, 12: 1944. 1954.

An ethylenediamine tetraacetate titration method is described for the routine analysis of Ca in plant materials, milk, rations, grains, feces, and other substances such as fertilizers in which inorganic phosphate interferes with the titration. Murexide is used as the indicator.

An anion exchange resin in the chloride form in a small column is used to remove interfering anions. The results obtained by the proposed procedure compare favorably in accuracy and precision to those of the classical oxalate permanganate method, and the method is much less time-consuming. In an over-all evaluation the ethylenediamine tetraacetate values were slightly lower than the oxalate values. The average difference, 0.024, is nearly equal to the average standard deviation of the oxalate method which was ±0.023. B. H. Webb

122. Determination of ionized calcium and magnesium in milk. G. CHRISTIANSON, R. JENNESS, and S. T. COULTER, Depts. of Agr. Biochem. and Dairy Husbandry, Univ. of Minn., St. Paul. Anal. Chem., **26**, 12: 1923. 1954.

A method is presented for determination of "apparent" Ca and Mg ion concentrations when these ions exist in equilibrium with several dissolved and colloidal complexes. A cation exchanger such as IR-100 or IR-120 is equilibrated against milk and the amounts of Ca and Mg that can be bound to the resin are determined. The quantities bound are converted to concentrations of Ca and Mg ions by comparison with the amounts bound by the exchanger in equilibrium with standard solutions containing Na, K, Ca, and Mg as the chlorides. Standard curves are prepared with concentrations of competing cations at the same level as in milk and at varying levels of Ca and Mg.

Apparent concentrations in skimmilk at 22°-25° C. are 2.0 to 2.3 mM of Ca and 0.82 to 0.85 mM of Mg per liter. This represents only a fraction of these elements in milk since there is a total of about 30 mM of Ca, about 10 mM of which is diffusible and 2 mM is ionized. Corresponding figures for Mg are 5 mM total, 3 mM diffusible and 0.8 mM ionized. Ion concentrations are strongly influenced by pH adjustment, addition of citrate, and previous heat treatment. Reproducibility of the method is good but in its present state of development it is not suitable for rapid routine determinations. B. H. Webb

123. Determination of solids-not-fat by lactometric methods. L. M. LAMPERT, Sacramento, Calif. Milk Dealer, **44**, 2: 49. 1954.

Official Methods now contain directions for determining the total solids content of milk by use of the lactometer. However, the sp.g. readings made at different laboratories differ and the calculated solids values often differ widely from galvimetric results on the same sample. The methods proposed by several research workers for correcting these variations are discussed. C. J. Babcock

124. Rapid, accurate test for S.N.F. W. J. JENNINGS and N. P. TARASSUK, Univ. of Calif., Davis. Milk Dealer, **44**, 3: 64. 1954.

The three general methods for determining total solids or solids-not-fat include the A.O.A.C. procedure, the Mojonnier total solids test, and indirect methods. The indirect methods are based on the relationship between the solids-not-fat and the fat content, the specific gravity of the milk or the amount of an individual solids constituent, such as protein. It is evident that only one method will be reasonably accurate and suitable to all kinds of milk under conditions where a representative sample can be obtained. The need for a gravimetric method for the direct measurement of total solids is indicated. Data are presented showing modifications of the Mojonnier total solids test directed toward shortening the time required to conduct the test. C. J. Babcock

DAIRY ENGINEERING

125. Valve for controlling the flow of milk and the like. BOLES B. IVANEK. U. S. Patent 2,695,156. 5 claims. Nov. 23, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 4: 766. 1954.

An easily demountable valve of sanitary construction, consisting of a valve which is pressed against when pressure is applied by a screw to the valve through a diaphragm. R. Whitaker

126. Application of the freeze-drying process in food preservation. H. WEISER, Dept. of Bacteriol., Ohio State Univ., Columbus. Milk Products J., **45**, 12: 15. 1954.

The author reviews the present status of the "drying by sublimation" or "freeze-drying" process. The objectives and principles involved in the dehydration of frozen materials are presented.

Some of the prolems involved, such as pretreatment of product, stability of final product, etc., are discussed briefly. This process which has been applied to numerous products such as fruit juices, meats, and blood plasma is relatively new and is limited at present by cost. Estimated cost of drying foods on a production scale, including fixed charges, is about 3¢ per lb. of water. J. J. Janzen

DAIRY PLANT MANAGEMENT AND ECONOMICS

127. New tax savings through depreciation. J. C. BEDFORD, Armstrong Coll., Berkeley, Calif. Milk Dealer, **44**, 3: 54. 1954.

Milk plant management has a choice of three methods of determining the rate of depreciation on plant, delivery and office equipment, and buildings. The straight-line method calculates the useful life of plant equipment and deducts the cost in equal annual installments over the anticipated useful life of the equipment. With the declining balance method, the tax savings are greater in the early years of the useful life of the milk plant's equipment, and if the depreciated equipment is sold before

its useful life has expired, a milk plant can end up with a large over-all tax saving. The sum-of-the-digits depreciation method is more complicated than either of the other two methods and does not give as much depreciation the first year, but, in a three-year span the total deductions are greater. Examples of calculating by each method are given. C. J. Babcock

128. Does paper packaging pay in small plants? M. K. CHRISTIANSEN and E. F. KOLLER, Univ. of Minn., St. Paul. Minn. Farm Business Notes, 357, 2. 1954.

A cost study of bottling fluid milk in glass and paper containers was made in three dairies whose daily volume was 2,000 qt. or less. Costs of bottling milk in glass were 3.6¢, 3.3¢, and 2.8¢ per qt. compared with 6.6¢, 6.1¢, and 7.9¢ per qt. when paper cartons were used. It was calculated that at the best operating volume, cost in paper cartons would be 5.75¢ per qt. in 2 plants and 5¢ in the other. It was concluded that selling milk in glass bottles should give these local plants a price advantage over milk brought in from outside sources; however, the smaller dairies would be at a disadvantage using paper cartons. A. W. Rudnick, Jr.

129. Can we sell more milk with quantity discount pricing? J. D. HELMBERGER and E. F. KOLLER, Univ. of Minn., St. Paul. Minn. Farm Business Notes, 357, 1. 1954.

Under a quantity discount pricing plan for route-delivered fluid milk, the customer pays one price for the first qt. delivered and a lesser price for subsequent qts. delivered at the same time. After a study of delivery costs in the Minneapolis area, it was thought feasible to increase the present 4¢ differential. A survey of 1,198 households in the market was made to determine consumer reaction to a 10¢ spread. On the basis of the data obtained, it was calculated that route delivery volume would be increased 15.2% over current volume. A 10¢ differential was introduced by one firm in Des Moines, Iowa, and retail route volume increased 13% over the corresponding period of the previous year, a remarkably close agreement to the calculated increase. Advantages and disadvantages of this and other plans are discussed.
A. W. Rudnick, Jr.

130. Labor market competition. T. D. LEATHERS. Milk Plant Monthly, **43**, 11: 25. 1954.

The decreasing number of young college graduates entering the dairy field may cause the industry to face a crisis because of its failure to obtain competent career men. Some segments of the dairy industry, mainly associations and colleges, are now attacking the problem through scholarships, etc. Programs underway at Rutgers, Georgia, Texas, Indiana, and in other parts of the country are discussed. Several means of attracting and keeping qualified employees and executives in the industry are suggested. C. J. Babcock

131. Budget for future profits. T. R. EBELL, Miller-Flounders Dairy Inc., Chester, Pa. Milk Plant Monthly, **43**, 12: 31. 1954.

The advantages of a budget plan for future profits or for coordinated operation of a business are discussed in detail. C. J. Babcock

132. Town bans vending. ANON. Milk Plant Monthly, **43**, 11: 36. 1954.

Overriding the vigorous objections of members of the milk industry, the Township Committee of Union, N. J., a town of 50,000 persons located southeast of Newark, voted to prohibit the erection or use of milk vending machines in that community. It is reported that the ordinance was motivated by a milk vending machine being located either on or adjacent to a public sidewalk in such a manner as almost to become a public nuisance, and the desire of some milk retailers in Union to eliminate the type of competition offered by the automatic merchandising of milk. An amendment can be made to the ordinance that would permit the installation of milk vending machines in schools and industrial plants. C. J. Babcock

FEEDS AND FEEDING

133. Grasslands improvement—a vast profit potential. Part I. The warmer humid region by G. W. BURTON. **Part II. The cool humid region** by H. B. SPRAGUE. **Part III. The great plains region** by J. R. HARLAN. J. Agr. Food Chem., **3**: 23. 1955.

The article is an interpretive survey of grassland management, its progress and potentialities in the northeast, southeast, and great plains sections of the country. S. Patton

134. Torula Yeast. ANON. J. Agr. Food Chem., **3**: 17. 1954.

The development of Torula yeast as a human and animal food ingredient is traced and evidence regarding its expanded production and usage is presented. A flow sheet for production of food yeast from sulfite liquors is given. The merits of Torula and Saccharomyces yeasts as high protein and B-vitamin supplements for mixed animal and poultry feeds are discussed briefly. S. Patton

135. Studies on jola or jowar (Andropogon sorghum) as cattle fodder. II. Chemical composition of white variety of jola at different stages of growth. H. C. LAKKE GOWDA. Indian J. Dairy Sci., **7**, 19. 1954.

The results show that as the plant reaches the full bloom stage, there was a progressive increase in organic matter, crude fiber, and nitrogen-free extract and a decrease in crude protein, ether extract, total ash, Ca, Mg, and P.
W. C. van der Zant

GENETICS AND BREEDING

136. The elimination of Trichomonas foetus from infected semen by storage in the presence of glycerol. L. P. Joyner, Weybridge. Vet. Record, **66**, 47: 727. 1954.

Previously Joyner and Millar (1950) demonstrated that normal artificial semen dilution and storage had no effect on *T. foetus*. This experiment was designed to study the effect of glycerol and freezing to –79° C. in the presence of glycerol on trichomonads. Trichomonads obtained from preputial washings of an infected bull and from cultures of an isolated strain were added to semen or seminal plasma. Egg yolk citrate diluent and 10% milk powder in citrate buffer were used. Several experiments tested the survival of trichomonads under various glycerol concentrations and time and temperature conditions. Freezing to –79° C. in the presence of 10% glycerol killed trichomonads and is the most satisfactory method of treating infected semen. Storing overnight at +5° C. in 20% glycerol also eliminated *T. foetus*. R. P. Niedermeier

137. Symmetrical alopecia in cattle. J. R. Holmes, Vet. Lab. of Bristol, and G. B. Young, Animal Breeding Research Organization, Edinburgh. Vet. Record, **66**, 46: 704-706. Nov., 1954.

Recent observation of alopecia in 2 Friesian herds is reported. Four animals were affected and all were born with a normal hair coat. Loss of hair began at 6 wk. to 6 mo. of age and progressed to complete baldness. Hair loss began on head, neck, back, and hind quarters and symmetrically progressed downward. Skin showed no irritation and histological examination indicated an irreversible condition. Post mortem examination disclosed no visceral or thyroid abnormalities. Pedigree analyses of affected heifers and their normal sibs suggest that an autosomal recessive gene is involved. R. P. Niedermeier

138. Dispensing refrigerator for artificial insemination. M. M. Hornish (assignor to The Curtiss Candy Co.). U. S. Patent 2,695,729. 2 claims. Nov. 30, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 5: 953. 1954.

An insulated box containing a compartment in which tubes of the product are maintained in an upright position. Refrigeration is supplied by ice cubes. A temperature modulating device before the tubes provides a relatively low constant temperature. R. Whitaker

139. Bacterial contaminants in buffalo semen. I. N. Mahmoud. Indian J. Dairy Sci., **6**, 197. 1953.

A discussion of the bacterial contaminants of buffalo semen is presented. The author recommends the addition of either 6 drops of a solution of penicillin (40,000 I.U./ml.) or 3 drops of a 0.1% solution of sulphanilamide to each semen sample collected in hot climates. W. C. van der Zant

140. Observations on some Indian cattle. V. Cross breeding for milk production. K. C. Sen, A. J. Lazarus, M. C. Rangaswamy, and C. P. Anantakrishnan. Indian J. Dairy Sci., **6**, 223. 1953.

A discussion on the cross breeding of *Bos taurus* with *Bos indicus* for milk production is presented. Cross-breds yielded more milk, produced milk for a longer period, had shorter service and dry periods, delivered their calf at an earlier age and had a longer productive life than their Zebu ancestors. An increased incidence of foot and mouth disease and abortion with an increase in Ayrshire blood is indicated. W. C. van der Zant

HERD MANAGEMENT

141. Can vs. tanks. P. E. Johnson, H. C. Olson, and R. L. Van Gunten, Dept. of Dairying, Oklahoma A and M Coll., Stillwater. Milk Plant Monthly, **43**, 11: 10. 1954.

The results of this study show that the standard plate counts on the milk handled by the bulk system were lower than those on the milk handled by the can system. There were no significant differences in the coliform counts on the raw milk or in the standard plate counts on the pasteurized milk attributable to the system of handling the milk. With the can system, the calculated loss was 0.32 lb. of milk per can. There were no differences in the flavor of the pasteurized milk processed from raw milk produced by the 2 systems. C. J. Babcock

142. Calf feeder pail assembly. H. E. Meltzer (assignor to Allover Manufacturing Co.). U. S. Patent 2,695,007. 4 claims. Nov. 23, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 4: 729. 1954.

A nipple, inserted through the side of a pail, is turned downward inside so that calf food is drawn from the bottom of the pail. R. Whitaker

143. Control device for milking machines. A. G. Perkins. U. S. Patent 2,696,194. 17 claims. Dec. 7, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 1: 100. 1954.

A description is given of a device located in the hose line on a milker between the teat cups and the milk reservoir which prevents restriction of hose when the milk is flowing, but which allows contraction of the hose when the milk flow ceases. R. Whitaker

144. Vacuum line with trap for milking machines. E. Domingo. U. S. Patent 2,696,193. 1 claim. Dec. 7, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 1: 100. 1954.

A jar trap is placed in the suction line between the milker and the individual stall or

stanchion petcocks, to prevent flow of milk and liquid impurities in either direction between the milker and the pipeline. R. Whitaker

145. Milking table. F. G. Hill. U. S. Patent 2,696,801. 7 claims. Dec. 14, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 2: 308. 1954.

A two-level milking stall, with the milk receiver located on a level lower than the cow. R. Whitaker

ICE CREAM

146. Selling through food stores. D. J. Cook, Ice Cream Merchandising Inst., Wash., D. C. Ice Cream Rev., **38**, 5: 48. 1954.

Several suggestions for increasing ice cream sales through grocery stores are detailed. W. J. Caulfield

147. Controlling delivery costs through realistic pricing. F. H. Bahil, Frechtling Dairy Co., Cincinnati, Ohio. Ice Cream Rev., **38**, 5: 42. 1954.

A discount schedule for ice cream based upon daily purchases has been used successfully at the Frechtling plant in Cincinnati for a period of over a year. Under this plan the discount schedule does not apply to 5 and 10¢ novelties. The new system provides for a more realistic pricing of ice cream and it has been an effective method of eliminating special deliveries. Each retail dealer knows exactly the cost of his ice cream at the time of purchase, and is in a position to control costs by ordering less frequently but in larger volume. W. J. Caulfield

148. How important are stick confections to your business? C. O. Lund, C. O. Lund and Assoc., Inc., Chicago, Ill. Ice Cream Rev., **38**, 5: 43. 1954.

The ice cream stick confection business today represents 91,000,000 gal. of product per year with a value to the dealer of $37,000,000. It is estimated the volume of ice cream and related products now being sold in the form of bar confections could easily be doubled. The author makes several suggestions for increasing sales. W. J. Caulfield

149. Ice cream confection. A. F. Hoff. U. S. Patent 2,695,237. 2 claims. Nov. 23, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 4: 784. 1954.

The tendency of ice cream cones to split vertically and break irregularly as consumed is eliminated by molding the cone with a series of closely spaced horizontal rings on the outside of the cone. As the cone is consumed, it fractures horizontally along the thin lines formed between the rings. R. Whitaker

150. Ice cream sandwich package. F. A. Russell (assignor to Russell Corp.). U. S. Patent 2,695,126. 7 claims. Nov. 23, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 4: 759. 1954.

A package is described consisting of lined corrugated paper, suitable for ice cream sandwiches. R. Whitaker

151. Frozen food merchandising cabinet having means to prevent condensation on lids. H. F. Luecke (assignor to Southern Dairies, Inc.). U. S. Patent 2,696,087. 7 claims. Dec. 7, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 1: 73. 1954.

Condensation on refrigerated cabinet lids is prevented by forcing a stream of air through a conduit at the back of the cabinet and directed across the lid through an outlet at the back and above the lid. R. Whitaker

152. Ice cream cup. S. C. Graham. U. S. Patent 2,697,041. 2 claims. Dec. 14, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 2: 367. 1954.

An individual cup-shaped edible ice cream container, with tapered sides to permit nesting, and a trough-like rim around the top for collecting melted ice cream. Rigidity is provided through the use of fins within the trough and the lower portion of the cup. R. Whitaker

153. Some factors affecting gelation of frozen egg yolk. A. Lopez, C. R. Fellers, and W. D. Powie, Dept. of Food Technol., Univ. of Mass., Amherst. J. Milk and Food Technol., **17**, 11: 334. 1954.

The pH of fresh egg yolk was not affected by freezing and frozen storage. Changing the pH of yolks, cooking at different temperatures and time intervals, diluting and dehydrating the yolks previous to freezing did not inhibit gelation.

Salt added to yolk prior to colloid milling, freezing and frozen storage, produced a yolk with a higher degree of gelation as compared to colloid milled samples with no salt added.

The various emulsion stabilizers and destabilizers tested had no appreciable effect on inhibiting gelation, while sugar, salt, and glycerol partially prevented gelation.

Eggs subjected to sonic and to supersonic vibrations previous to freezing and frozen storage, showed a marked degree of gelation upon thawing. H. H. Weiser

MILK AND CREAM

154. Studies on vitamin A in milk. VII. Effect of processing on the stability of vitamin A in fortified milk. N. B. Shroff, K. M. Narayanan, C. P. Anantakrishnan, and K. C. Sen. Indian J. Dairy Sci., **7**, 40. 1954.

In certain seasons the vitamin A content of Indian milk is very low because most of the cows and buffaloes receive very little green feed except during the monsoon season. The practice of using reconstituted skimmilk to reduce the fat content of buffalo milk produces a milk of low vitamin A potency. Furthermore, prolonged boiling of milk is common in the preparation of indigenous milk products. The vita-

min A preparations which were added to fortify milk were stable to pasteurization and boiling. The vitamin A in buffalo milk behaved the same as in cow's milk. W. C. van der Zant

155. Apparatus for manufacturing whipped cream. F. F. SUELLENTROP (assignor to Lemay Machine Co.). U. S. Patent 2,695,123. 4 claims. Nov. 23, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 4: 758. 1954.

Details are given for a device for closing cans partially filled with cream and under a predetermined pressure of gas. R. Whitaker

156. Liquid dispensing machine. L. M. HARVEY. U. S. Patent 2,695,567. 12 claims. Nov. 30, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 5: 913. 1954.

A sanitary type dispenser for milk and other liquid food products, consisting of a single service collapsible bag with a flexible inlet on top and outlet on the bottom, within a refrigerated chamber and fed from a bulk can of product also adequately refrigerated. Insertion of a coin starts the following automatic serving cycle, with the bag holding the volume of milk to be dispensed: (1) place cup under outlet tube, (2) open pinch valve on outlet tube, (3) collapse bag, (4) close outlet valve, (5) open inlet pinch valve and fill bag, (6) close inlet valve, thereby returning the device to original setting. R. Whitaker

157. Beverage dispensing apparatus. P. J. DANIELS. U. S. Patent 2,695,121. 7 claims. Nov. 23, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 4: 757. 1954.

Milk or other fluid is dispensed from an inverted milk can having a leakproof lid through a flexible tube by means of a rotary tube compressing device to be delivered into a cup for immediate consumption. The can and cup-holding chamber are contained in an insulated cabinet. R. Whitaker

158. Beverage dispensing apparatus. P. J. DANIELS. U. S. Patent 2,695,117. 8 claims. Nov. 23, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 4: 756. 1954.

Milk or other fluid is dispensed from a milk can by intermittently compressing upward a flexible tube extending to the bottom of the can. R. Whitaker

159. Dispensing carton with gable top. C. H. DIXON (assignor to Ex-Cell-O Corp.). U. S. Patent 2,695,745. 1 claim. Nov. 30, 1954. Offic. Gaz. U. S. Pat. Office, **688**, 5: 957. 1954.

A method of folding the top of a Pure-Pak paper milk bottle, to form a protected pouring lip. R. Whitaker

MILK SECRETION

160. Effect of age on total milk yield and length of lactation period in the Egyptian buffalo. M. T. RAGAB, A. A. ASKER, and M. S. GHAZY. Indian J. Dairy Sci., **6**, 181. 1953.

The data were taken from 923 lactations of which 345 were first lactations; the rest ranged between the 2nd and 6th lactation. Buffaloes calved for the first time at an average age of 40.2 mo. Buffaloes could calve for the first time without a serious reduction in milk production at 27-30 mo. of age. The milk yield increased with age up to the third lactation and then declined. Maximum production was reached at an age of 6-7 years. The age of first calving did not seriously affect the lactation period.

W. C. van der Zant

161. Effect of season of calving, dry period, and calving interval on milk yield and lactation period of Egyptian buffaloes. M. T. RAGAB, A. A. ASKER, and M. S. GHAZY. Indian J. Dairy Sci., **7**, 8. 1954.

The results were taken from data comprising 929 lactations of 345 buffaloes recorded during 1940-1950. Fall and winter calvers had slightly higher milk yield and a longer lactation period than those calving in summer and spring. The average current dry period was 209.6 d. There was a negative correlation between current dry period and milk yield. The average preceding dry period was 210.6 d. This dry period was an important cause of variation in the total milk yield. The average length of calving interval was 541.7 d. There was a positive correlation between milk yield and calving interval. It also was found that the month of calving, and preceding dry period had a significant influence on the lactation period.

W. C. van der Zant

NUTRITIVE VALUE OF DAIRY PRODUCTS

162. Milk—vitality for all ages. H. RIDDELL. Univ. of Vt., Burlington. Milk Dealer, **44**, 3: 59. 1954.

Milk provides more food value for the money than any other food. It has been figured that to buy calcium, protein, and vitamins in a qt. of milk would entail the purchase of nearly 60¢ worth of other food. At current prices, the price of an oz. of protein in milk is less than ½ the cost in steak, while in cottage cheese it is only 1/3 as much. Milk in terms of all-'round nutritive value is our most economical food. The price of a qt. of milk delivered today is about 80% above prewar. The average price of all other foods has risen 130% in the same time. In 1939, the individual worker averaged about 11½ min. work to earn a qt. of milk. Today a little over 8 min. labor will purchase the same amount. C. J. Babcock

PHYSIOLOGY AND ENDOCRINOLOGY

163. The effect of feeding thyroprotein on the composition of milk of Egyptian buffaloes. A. A. Asher, M. T. Ragab, and T. H. Kamal. Indian J. Dairy Sci., 7, 36. 1954.

Four mature buffaloes were given 15 g. of Protamone per head per day for 18 wk. A similar group which did not receive Protamone was used as control. The influence of feeding thyroprotein on the protein, total solids, solids not fat, and chlorine content, and specific gravity of buffalo milk was studied. No significant difference between test and control group was found. W. C. van der Zant

164. Effect of feeding iodinated casein on growth and food utilization by calves. K. C. Sen, G. K. Murthy, B. N. Premachandra, N. N. Dastur, and S. C. Ray. Indian J. Dairy Sci., 6, 201. 1953.

Sindhi and Gir heifers were fed 2 g. of iodinated casein per 100 lb. weight for 28 wk. The growth rate of the animals receiving iodinated casein was 33-55% lower than that of their respective controls. The test group showed the same capacity for digesting the food and consumed the same amount of crude protein and total digestible nutrients per 100 lb. weight as the control group. There was a lower retention of N and a larger secretion of it in the urine by the animals receiving the iodinated casein. W. C. van der Zant

165. A technic for aspiration liver biopsy in dairy cattle. J. F. Bone, Corvallis, Ore. North Am. Veterinarian, 35, 10: 747. 1954.

A liver biopsy instrument consisting of a modified aspiration biopsy needle is detailed. The surgical technique for liver biopsy is described. Liver cores obtained averaged approximately 1 g. The author has performed this biopsy on 64 dairy cattle ranging from 3 d. to 13 yr. of age with no unfavorable results. R. P. Niedermeier

SANITATION AND CLEANSING

166. The use of radioactive phosphorus to measure the amount of milkstone deposited on rubber, pyrex glass, and tygon tubings. M. W. Cucci. Milk Plant Specialties Corporation, Rochester, N. Y. J. Milk and Food Technol., 17, 11: 332. 1954.

A study of the accumulation of milkstone on the surface of milk equipment has been made. Chemical and physical factors were considered as contributory to milkstone formation and high bacterial counts.

Approximately 0.1 ml. of radioactive phosphorus (P^{32}) in the form of phosphate was added to a solution of 225 ml. of homogenized milk and 45 ml. of 10% trisodium phosphate dodecahydrate. A 1-ml. aliquot had an activity of 2,500 c.p.m. per ml.

The results showed that rough and porous surface of rubber had twice as much milkstone deposit as glass. There was no deposit on tygon during the given time exposure. H. H. Weiser

167. Contamination of rubber milk tubes of milking machines as affected by deterioration of the inside surfaces. T. J. Claydon, Kan. Agr. Expt. Sta., Manhattan. J. Milk and Food Technol., 17, 12: 359. 1954.

A study was made on the susceptibility of new and used rubber tubing to bacterial contamination. Used tubing showed a log ave. count 15 times as great as the bacterial counts on new tubes. Upon sanitization and dry storage, the log average count of the used tubes was 12 times as great as that of the new tubes. When lye storage was substituted for dry storage, the bacterial counts were markedly lower in both types of tubes.

The author suggests that a potential problem exists in milking machine sanitation. Modifications in rubber composition or suitable plastic milk tubes may help to minimize the problem. H. H. Weiser

168. How you save with C-I-P lines. G. Nelson, Ore. State Coll., Corvallis. Milk Plant Monthly, 43, 12: 29. 1954.

In-place cleaning reduces labor requirements 77%. Data are presented showing the labor saved in plants using the same cleaning procedure and in plants cleaning only cold milk lines. Labor for in-place cleaning requires 1/4 to 1/5 as much time as daily disassembling, hand cleaning, and reassembling the pipelines. In addition to savings in labor there is less chance to damage pipelines and this permits the use of thinner, less expensive lines. C. J. Babcock

169. Convertible can and case washer. H. D. Lathrop and E. E. Hageline (assignors to The Lathrop-Paulson Co.). U. S. Patent 2,696,822. 4 claims. Dec. 14, 1954. Offic. Gaz. U. S. Pat. Office, 689, 2: 313. 1954.

A washer designed to wash milk cans and milk bottle cases. R. Whitaker

170. Milk can scrubber. H. R. Pendleton. U. S. Patent 2,696,625. 3 claims. Dec. 14, 1954. Offic. Gaz. U. S. Pat. Office, 689, 2: 265. 1954.

A brush for cleaning all of the inside surface of a milk can. The brush, formed to fit the contour of the can, is mounted on a rotating shaft. To permit inserting and withdrawing the brush through the neck of the can, the brush is made in sections which are pivoted on the shaft. R. Whitaker

Year In, Year Out—
Always Dependable

UNIFORMITY IS IMPORTANT! Leading agricultural schools and colleges agree that the regular weekly use of FLAV-O-LAC FLAKES assures uniformity of smoothness, aroma and flavor in fermented milk products.

YOGHURT CULTURE

FLAV-O-LAC FLAKES (shown) produce a quart of the finest starter on a single propagation. Single bottles $2.00.

New Cheddar Cheese Culture
Flav-O-Lac Flakes S. durans
$2.00 (plus postage)

THE DAIRY LABORATORIES
A DIVISION OF DALAKE ASSOCIATES
123rd & Locust Sts., Phila. 3, Pa.
BRANCHES
New York Baltimore Washington
See our catalog in Dairy Industries Catalog

"HANSEN'S"
DAIRY PREPARATIONS
For High Quality Dairy Products

- ☐ CHEESE RENNET AND COLOR
- ☐ COTTAGE CHEESE COAGULATOR
- ☐ ANNATTO BUTTER COLOR
- ☐ DANDELION BUTTER COLOR
- ☐ CERTIFIED BUTTER COLOR
- ☐ STARTER DISTILLATE
- ☐ ICE CREAM COLOR
- ☐ LACTIC FERMENT CULTURE
- ☐ CULTURE FLASKS
- ☐ CULTURE CABINETS
- ☐ TESTING SOLUTIONS
- ☐ ODORLESS TYPE DAIRY FLY SPRAY

Check and Mail for Literature

CHR. HANSEN'S LABORATORY, INC.
MILWAUKEE 14, WIS.

Reliable Service Since 1878.

Mention

Journal of Dairy Science

When Answering Advertisements

THIS HELPS BOTH THE ADVERTISER AND YOU

Your advertisement is being read in every State and in 45 Foreign Countries

AMERICA'S MOST COMPLETE LINE OF

Job-Fitted

ALKALINE CLEANERS

★BREADTH ★DEPTH

KLENZADE

FOR *Economy* CLEANING

For Hard Water

KLENZADE HC-55

Specially compounded with a synergist which accelerates detergency and eliminates water hardness cleaning troubles. Rinses clear and clean; leaves no spots or streaks. Prevents waterstone and scum build-up. Heavily fortified with top-efficiency multipurpose polyphosphates and superior wetting agents. Klenzade HC-55 will definitely save cleaning time and material — the positive answer to your hard water problems. HC-55 . . . a star item in our famous line of "job-fitted" alkaline cleaners.

Super Wet KLENZADE HC-7

FOR YOUR TOUGHEST CLEANING JOBS

Somewhere, in every plant, there's a real tough cleaning job that wastes time and materials . . . and won't respond to ordinary cleaners. Klenzade Super Wet HC-7 is designed expressly for this purpose. Removes heavy soil and high fat concentrations, suspends and sequesters hard water minerals. Super Wet is an outstanding achievement in "balanced" detergency . . . a super 3-power attack on soil with polyphosphates, alkalis, and wetting agents. Another star in our famous "job-fitted" alkaline cleaner line.

"FIRST IN CLEANING CHEMICALS"

KLENZADE KLENZADE PRODUCTS, INC.

BELOIT WISCONSIN

Copies of the

20-YEAR INDEX

covering Volumes I to XX, inclusive are available at the following prices:

•

MEMBERS

Cloth Bound $2.35

Paper Bound 2.00

•

NON-MEMBERS

Cloth Bound $5.50

Paper Bound 5.00

DIFCO

Bacto-Dehydrated Media for Microbiological Assay of

VITAMINS and AMINO ACIDS

These media contain all the necessary nutriments for the growth of specified test organisms for the microbiological assay of vitamins and amino acids except for the component under assay. These basal media require only the addition of specified increasing amounts of the vitamin or amino acid being assayed to obtain a growth response which may be measured by acidimetric or turbidimetric methods for the construction of standard curves. The vitamin or amino acid content of the material under assay is determined by adding appropriate concentrations of the test substance to the basal medium and comparing the growth response obtained with that of the standard.

BACTO-RIBOFLAVIN ASSAY MEDIUM
BACTO-NIACIN ASSAY MEDIUM
BACTO-THIAMIN ASSAY MEDIUM
BACTO-PANTOTHENATE ASSAY MEDIUM
BACTO-B_{12} ASSAY MEDIUM USP
BACTO-CS VITAMIN B_{12} AGAR
BACTO-FOLIC ACID ASSAY MEDIUM
BACTO-PYRIDOXINE ASSAY MEDIUM
BACTO-BIOTIN ASSAY MEDIUM
BACTO-CHOLINE ASSAY MEDIUM
BACTO-CF ASSAY MEDIUM
BACTO-TRYPTOPHANE ASSAY MEDIUM
BACTO-LEUCINE ASSAY MEDIUM
BACTO-METHIONINE ASSAY MEDIUM
BACTO-LYSINE ASSAY MEDIUM
BACTO-ISOLEUCINE ASSAY MEDIUM
BACTO-ARGININE ASSAY MEDIUM
BACTO-TYROSINE ASSAY MEDIUM
BACTO-CYSTINE ASSAY MEDIUM

The method employed in carrying stock cultures of the test organisms and preparing the inoculum for microbiological assay is important. The following media have been developed especially for carrying stock cultures and for preparation of the inoculum:

BACTO-MICRO ASSAY CULTURE AGAR
BACTO-MICRO INOCULUM BROTH
BACTO-B_{12} CULTURE AGAR USP
BACTO-B_{12} INOCULUM BROTH USP
BACTO-NEUROSPORA CULTURE AGAR

BACTO-VITAMIN FREE CASAMINO ACIDS, dehydrated, is an acid hydrolysate of vitamin free casein prepared especially for laboratories investigating microbiological assay of vitamins.

Descriptive literature available upon request

DIFCO LABORATORIES
DETROIT 1, MICHIGAN